Bachelor's project

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biological Sciences Ålesund

Candidate number: 10014 og 10015

Bachelor Thesis

Optimisation of a Protocol for the Analysis of Cell Cycle in Chinook Salmon Embryonic Cells (CHSE-214) Using Flow Cytometry: Analysis of Cell Cycle in Response to Thermal Stress

Bachelor's project in Bioteknologi

Supervisor: Bjørnøy, Gro Audveig Hagen. Cao, Yanran

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Abstract

This report presents the process of the optimisation of a protocol for the study of the cell cycle in Chinook salmon embryonic cells (CHSE-214) using flow cytometry. The study resulted in the production of an optimised method for the study of the cell cycle in CHSE-214 cells using a PI/RNase staining solution.

This report also presents a study of the theoretical and practical views of the effect of temperature on CHSE-214 cells by analysing their cell cycle development and variations. For the study, the cells were cultivated at 20 °C (CHSE-214 control) and at 26 °C (CHSE-214 stressed) and stained with propidium iodide (PI) for further proliferation analysis using flow cytometry. Conclusions on the results could not be drawn due to the low amounts of parallels used in the study.

List of Abbreviations

ATP	adenosine triphosphate
CDKs	cyclin-dependent kinases
CPN	chaperonins
CSE	Chinook salmon embryonic cells
DAPI	nuclear and chromosome counterstain for fluorescence microscopy
DNA	deoxyribonucleic acid
EB	ethidium bromide
ER	endoplasmic reticulum
FBS	fetal bovine serum
FSC	forward scatter
GRPs	glucose-regulated proteins
HBSS	Hank's Balanced Salt Solution
HDG	Hsf1-dependent genes
HSF	heat shock factor
HSPs	heat shock proteins
IPNV	infectious pancreatic necrosis virus
LAF	laminar flow
NBD	nucleic acid database
PBS	phosphate-buffered saline
PI	propidium iodide
RNA	ribonucleic acid
SSC	side scatter
SMC	structural maintenance of chromosomes
TDA	tropodithietic acid

1. Introduction

The main focus of this report is to present an optimisation protocol for the study of the cell cycle in Chinook salmon embryonic cells (CHSE-214) using flow cytometry. Additionally, the report focuses on the study of cell cycle variations in CHSE-214 cells when exposed to stress.

CHSE-214 cells are susceptible to a wide range of fish viral pathogens. This cell line has been used for the growth and titration of infectious pancreatic necrosis virus (IPNV) from samples. It has also been used to carry out cell infection assays to determine the effect of tropodithietic acid (TDA)-exposed isolates [1].

Cells are sometimes stressed due to different factors such as exposure to toxins, mechanical damage or, as in this study, increase in temperature. Such changes in their environment cause the cells to develop specific strategies in order to deal with the stressful conditions.

Individual stress proteins have been identified and characterized from a wide variety of different organisms. Due to differences in their mode of regulation, the stress proteins are often divided into two major groups: heat shock proteins (HSPs) and glucose-regulated proteins (GRPs). In mammalian cells, the proteins whose synthesis increases after heat shock are referred to as HSPs, whereas the other major family, the GRPs, have been identified as showing increased expression in cells starved of glucose [2].

Cells from all organisms have developed a remarkably similar response to sudden increases in their normal growth temperature. This response, which was initially referred to as the heat shock response, is characterized by extremely rapid increase in the expression of HSPs [2].

Heat shock proteins ensure survival under stressful conditions, which if left unchecked would lead to irreversible cell damage and ultimately to cell death (Figure 1). Heat shock proteins have essential roles in the synthesis, transport, and folding of proteins and are often referred to as molecular chaperones [3].

Heat shock response involves transcriptional activation mediated by a transcription factor known as a heat shock factor (HSF). In unstressed cells, HSF is present in both the cytoplasm and the nucleus in a monomeric form that has no DNA binding activity. In response to heat shock and other physiological stresses, HSF assembles into a trimer and accumulates within the nucleus [4].

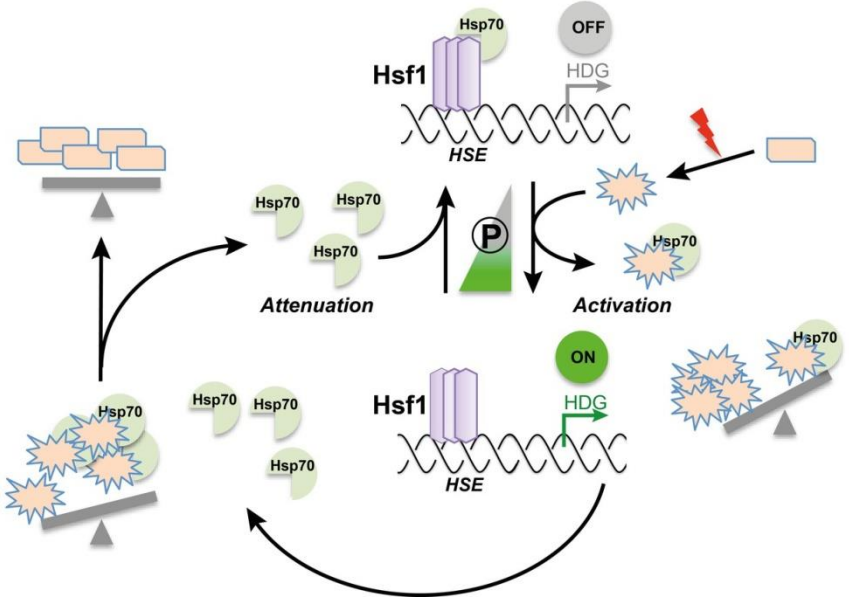

Figure 1. The chaperone titration model of the heat shock response [5]
Clockwise from top: The chaperone protein Hsp70 binds to the heat shock transcription factor Hsf1, repressing its transcriptional activity. Upon a sudden increase in temperature or other stresses (red lightning bolt), fewer proteins maintain their correct shape (rectangles); consequently, misfolded proteins (stars) accumulate in the cell. These misfolded proteins draw Hsp70 away from Hsf1, activating its transcriptional activity. As a result, more Hsf1-dependent genes (HDG) are expressed, leading to an increase in the number of chaperones and proteases, among them Hsp70, in the cell. The action of the chaperones and proteases ensures that proteins can be correctly folded again. This action also liberates Hsp70, which can then repress Hsf1. Middle: Hyperphosphorylation of Hsf1 (the width of the triangle represents the extent of phosphorylation) partially activates Hsf1 and sensitizes the regulatory feedback circuit.

1.1 Chinook salmon

Chinook salmon (*Oncorhynchus tshawytscha*) is probably the most enigmatic species of the Pacific salmon genus *Oncorhynchus*. Although least abundant, Chinook salmon are the most diversified in life history expression and show the widest geographic distribution. Although they are well-known for their potential body size (exceeding 45 kg), the species is classed as endangered today, Chinook salmon have been the subject of much debate and expense in fishery allocations and restoration efforts, including extensive investments in hatcheries over the past century. Recent genetic studies of Chinook salmon have increasingly focused on the molecular basis of phenotypic traits and the landscape distribution of putatively adaptive variation. New DNA sequencing methods make it possible to survey variation across tens of thousands of DNA base pairs and document specific molecular variations that are correlated with environmental variables and appear related to local adaptation [6].

1.2 Cell culture

Cell cultures have been widely used in laboratories for many years, with the aim to investigate and discover new properties from specific organism's cells.

Cell culture technique was first developed in the early 20th century as a method of studying animal cell behaviour in vitro. The principle of cell culture was established when embryologist Wilhelm Roux used warm saline to maintain a chicken embryo for several days, which established the principle of tissue culture [7].

Cell culture refers to the removal of cells from an animal or plant and their subsequent growth in a favourable artificial environment. The cells may be removed from the tissue directly and disaggregated by enzymatic or mechanical means before cultivation, or they may be derived from a cell line or cell strain that has already been established [6].

Primary culture refers to the stage in the culture after the cells have been isolated from the tissue and proliferated under the appropriate conditions until they occupy all the available substrate (i.e. until they reach confluence). At this stage, the cells must be sub-cultured (i.e. passaged) by transferring them to a new vessel with fresh growth medium in order to provide more space for continued growth [7].

After the first subculture, the primary culture becomes known as a cell line or subclone. Cell lines derived from primary cultures have a limited life span (i.e. they are finite), and as they are passaged, cells with the highest growth capacity predominate, resulting in a degree of genotypic and phenotypic uniformity in the population [7].

Normal cells usually divide only a limited number of times before losing their ability to proliferate, which is a genetically determined event known as senescence; these cell lines are known as finite. However, some cell lines become immortal through a process called transformation, which can occur spontaneously or can be chemically or virally induced. When a finite cell line undergoes transformation and acquires the ability to divide indefinitely, it becomes a continuous cell line [7].

1.2.1 CHSE-214

The CHSE-214 cell line originates from cells taken from the Chinook salmon (*Oncorhynchus tshawytscha*) embryo, which is susceptible to a wide range of fish viruses and in many instances replicates high titres [1].

The CHSE-214 cell line has been used for the growth and titration of infectious pancreatic necrosis virus (IPNV) from samples. It has also been used to carry out cell infection assays to determine the effect of tropodithietic acid (TDA)-exposed isolates [1].

1.3 Stress response – HSPs

Cells from all organisms can be exposed to many different conditions, including conditions that lie outside the optimal range for cell growth. This means that cells can easily be affected by varying temperature, oxygen concentration, salinity, and humidity, among many other factors.

In recent years, an understanding of the general roles of the major heat shock proteins (HSPs) in cell physiology has begun to evolve. HSPs are directly involved in the biogenesis of proteins from the time of synthesis as nascent chains until the assembly of multimeric complexes and have therefore been termed molecular chaperones [8].

Protein folding and the assembly of multimeric structures in vivo is not a completely spontaneous process but is facilitated by proteins called molecular chaperones. Molecular chaperones bind transiently and non-covalently to nascent polypeptides and unfolded or unassembled proteins, aiding in protein biogenesis in two general ways: they block non-productive protein–protein interactions, and they mediate the folding of proteins to their native state by sequestering folding intermediates, allowing the concerted folding by domains and assembly of oligomers. The two major groups of molecular chaperones are the 70-kDa family of HSPs (HSPs70s) and the 60-kDa family of HSPs (CPN60s) [8].

1.3.1 HSP70

HSP70s, which are found in all major cellular compartments of eukaryotes and every bacterium examined to date, bind partially unfolded proteins. They appear to bind nascent chains in the process of protein synthesis and completed polypeptides upon release from ribosomes. In addition, HSPs70s located in the mitochondria and endoplasmic reticulum (ER) play a critical role in translocation of proteins from the cytosol into those organelles by binding during the initial stages of translocation [8].

1.4 Cell cycle

Cell division is a very important process in all living organisms. During the division of a cell, DNA replication and cell growth take place. All of these processes (i.e. cell division, DNA replication, and cell growth) have to take place in a coordinated way to ensure correct division and formation of progeny cells containing intact genomes. The sequence of events by which a cell duplicates its genome, synthesises the other constituents of the cell, and eventually divides into two daughter cells is termed cell cycle [9]. The cell cycle consists mainly of five phases: G0, G1, S, G2, and M.

The regulation of the cell cycle must ensure that the events in each phase are fully completed before moving on to the next phase in the cell cycle. Thus, checkpoints for monitoring the integrity of DNA are strategically placed in late G1 and at the G2–M interface to prevent progression and propagation of mutated or damaged cells [9].

1.4.1 G1 phase

G1 (Gap 1) is the interval or gap between mitosis (M phase) and DNA synthesis (S phase). During G1 the cell is subject to stimulation by extracellular mitogens and growth factors. In response to the stimuli, the cell passes through G1 and proceeds with DNA synthesis in the S phase [10].

1.4.2 S phase

Chromosomal DNA is duplicated during a subportion of interphase S (synthesis). As the two daughter DNA strands are produced from the chromosomal DNA during S phase, they recruit additional histones and other proteins to form structures known as sister chromatids. The sister chromatids, in turn, become ‘glued’ together by a protein complex named cohesin. Cohesin is a member of the SMC (structural maintenance of chromosomes) family of proteins. SMC proteins are DNA-binding proteins that affect chromosome architectures. At the end of S phase, cells are able to sense whether their DNA has been successfully copied. They do this by using a

complicated set of checkpoint controls that are still not fully understood. For the most part, only cells that have successfully copied their DNA will proceed into mitosis [11].

1.4.3 G2 phase

The completion of DNA synthesis is followed by the G2 phase (Gap 2), during which cell growth continues and proteins are synthesized in preparation for mitosis [12].

1.4.4 M phase

Mitotic division mainly consists of four phases: prophase, metaphase, anaphase, and telophase. Chromatids are condensed into chromosomes during the prophase. These chromatids are aligned in the equatorial plate of the cell by the forming spindle apparatus. The kinetochore microtubules, which are connected to the centromeres of the chromosomes are contracted, generating tension on the centromere, which holds the two sister chromatids together at the anaphase. The tension leads to the cleavage of cohesion protein complexes in the centromere, separating the two sister chromatids and producing two daughter chromosomes. Thee daughter chromosomes are pulled towards the opposite poles by further contraction of the kinetochore microtubules during the final phase of the mitotic division, the telophase. After completing the M phase, the parent cell undergoes cytoplasmic division, known as cytokinesis, which results in two genetically identical daughter cells [14].

1.4.5 G0 phase

G0 refers to cells that are quiescent (temporarily or permanently out of cycle). The normal cell is dependent on external stimuli (mitogens or growth factors) to move it out of G0 and through the early part of G1. The cell responds to the external stimuli, which are communicated through a cascade of intracellular phosphorylation, by upregulating expression of the cyclins that associate with the cyclin-dependent kinases (CDKs) [10].

The duration of the cell cycle phases varies considerably in different kinds of cells. For a typical rapidly proliferating human cell with a total cycle time of 24 hours, the G1 phase might last about 11 hours, S phase about 8 hours, G2 about 4 hours, and M about 1 hour [12].

1.5 Flow cytometry

Flow cytometry is used to count and analyse the size, shape and properties of individual cells within heterogeneous population of cells. The cytometry data are highly suitable for quantification and can thus give a lot of information. In addition, the data can be analysed in depth by specific flow cytometry software programmes. Once a suspension of individual cells has been prepared for flow cytometry analysis from cells, tissues or organisms, the prepared sample is placed in the flow cytometer [14].

A flow cytometer has several key components: the sample moved into the flow cytometer as a fluid, lasers, optics that gather the light, detectors to sense the light, and a computer system to output the data into a form that can be analysed by the researcher (Figure 2). Once the sample has been placed in the flow cytometer, it will suck up the sample, mix it into a stream of saline solution, and lead the cell suspension through a narrowing channel, which causes the cells within the sample to line up in single file before they pass through the laser at the interrogation point. Each cell flows through the laser beam, at which point it can be analysed. As each cell passes, the laser beam will scatter light in multiple directions. The flow cytometer detects light scattered in a forward manner (forward scatter), and light scattered in a sideways matter (side scatter). The amount of forward scattered light for each cell is detected by a detector on the far side of the cell from the laser. Forward scatter is proportional to the size of the cell. The detector converts the scattered light into a voltage pulse, which is directly proportional to the amount of forward scattered light. The computer converts these data into a histogram plot, with the amount of forward scattered light on the x-axis and number of cells on the y-axis. The amount of side scattered light is detected by a detector located perpendicular to the path of the laser beam. The side scatter is proportional to the shape and internal complexity of a cell. Also, this scattered light is converted into a voltage pulse, just like the forward scattered light. By analysing the forward and side scattered data together, the researcher can understand a cell’s size, shape and

complexity. In addition, the data can allow the researcher to divide the heterogeneous population of cells into individual populations of varying size, shape and complexity. The capability of analysing multiple populations within a sample is the main strength of the flow cytometry experimental technique [14].

In addition, a flow cytometer can detect emitted light from excited fluorescent molecules, such as fluorescently labelled antibodies or fluorescent dyes or stains. The fluorophores are excited by the correct wavelength of the laser light when the cell passes through the laser beam. After excitation, the light emitted by the fluorophore is directed along a path with emission filters that allow for the detection of multiple different fluorophores emitting light in a cell. The flow cytometer can detect the presence of a fluorophore and quantify the relative amount of a fluorophore within a cell. Multiple fluorophores can be detected from the same sample. The data are then presented in the form of a histogram or a dot plot. Such data increase the power of flow cytometry analysis by enabling the researcher to analyse individual populations of cells within a heterogeneous mix of cells in the sample [14].

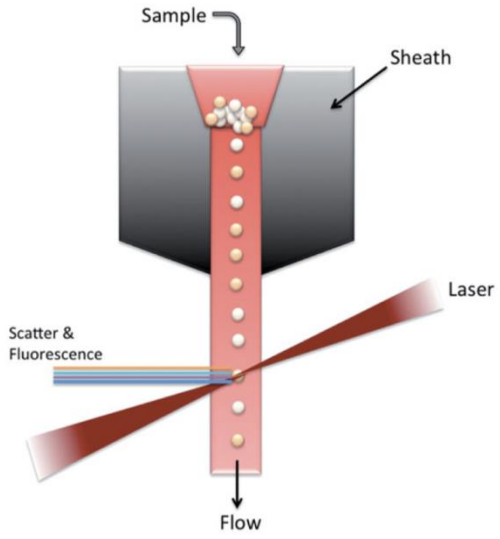

Figure 2. The basic components and working principle of a flow cytometer [15]

1.5.1 DAPI

DAPI (4',6-diamidino-2-phenylindole) is a type of fluorescent dye that can bind DNA strands robustly. The fluorescence can be detected by fluorescence microscope. DAPI can penetrate fixed cells (and living cells in high concentrations) as it can pass through the membrane. The molecular formula is $C_{16}H_{17}Cl_2N_5$, with 350.25 molecular weight, and CAS Number 28718-90-3 [16].

DAPI can bind the double-stranded DNA in the nucleus and produce 20 times stronger fluorescence than itself. The sensitivity for double-stranded DNA staining is many times greater compared with ethidium bromide (EB). Blue fluorescent cells would be visible under the microscope. The efficiency detected by fluorescence microscope is very high (almost 100%), and there are no side-effects for the living cells. DAPI staining is usually used in cell death detection. After staining with DAPI, detection with fluorescence microscope or flow cytometry is possible. DAPI is also used in nucleus staining and double-strand DNA staining in some cases [16].

The largest excitation wavelength for DAPI is 340 nm, and the largest emission wavelength is 488 nm. When DAPI binds with double-stranded DNA, the largest excitation wavelength is 360 nm, while the largest emission wavelength is 460 nm [16].

1.5.2 PI

Propidium iodide (PI) is a fluorescent vital dye that stains DNA and RNA. As PI binds to both DNA and RNA, the latter must be removed by digestion with ribonucleases. The content of DNA as determined by flow cytometry can reveal useful information about the cell cycle and the proteins involved in the regulation of the cell cycle. Cells in the G2 and M phases of the cell cycle have double the DNA content of those in the G0 and G1 phases. Cells in the S phase have a DNA content lying between these extremes. PI is detected in the orange range of the spectrum by using a 562–588 nm band pass filter. This reagent may be used to analyse cell cycle by flow cytometry [17].

2. Materials and methods

During the optimisation process both DAPI and PI/RNase was utilized when preparing and staining cells for samples. It was tested if the methods for DAPI and PI/RNase could stain CHSE-214 cells separately and together. For the stress experiment it was concluded that only PI/RNase would be used.

2.1 Cell culture in practice

The CHSE-214 cell culture preparation was carried out in a LAF (laminar flow) cabinet under sterile conditions. First, the medium was prepared (Appendix A1). A 500 mL flask of Leibovitz's L-15 Medium (Gibco of Life Technologies™, #31415-029) was used at 20 °C. A bottle (shot) containing 50 mL of fetal bovine serum (FBS, Gibco of Life Technologies™, #A3840401) was thawed for several minutes and introduced into the L-15 Medium. The FBS was used as a growth supplement for the cell culture because it contains a high concentration of growth factors. To complete the preparation of the medium, 0.5 mL of a 50 mg/mL Gentamicin (Gibco by Life Technologies ™, #15750-037) stock solution was added to the L-15 medium flask. Gentamicin is an antibiotic used to avoid bacterial infections.

2.2. Cell splitting optimisation

Initially, cell splitting was done by followed the method that had already been created (referred to in Appendix A2). A general overview of the steps performed in the cell splitting can be seen in figure 3. First, a 75 cm^2 cell flask (VWR by We Enable Science, #734-2313) was removed from an incubator set at 20 °C and placed under an inverted microscope. The microscope was used to view and assess the confluency of the cells. When the confluency was equal to or above 70%, the cells were deemed ready to be split. The flask was placed in an operational LAF cabinet, where the flask could be opened without the risk of introducing contaminating organisms. The old medium in the flask was removed and the flask rinsed twice with 5.0 mL of HBSS (HyClone™, #SH30588.02) to remove all traces of the old medium and thus prevent its interference in later steps. Then, 1.0 mL of Trypsin (Gibco of Life Technologies™, #25200-072)

was added to loosen the cells from the substrate. The flask was then removed from the LAF cabinet so that it could again be studied under the inverted microscope. Two new 75 cm^2 flasks were prepared by labelling them with descriptions of their content, passage, date, and owner. Thereafter, 17.0 mL of medium were added to the flasks there was added. After about 5 minutes, the flask was tapped slightly a few times and studied under the inverted microscope to evaluate the percentage of cells that had loosened from the substrate. When at least 80% of the cells had loosened, the trypsinization was deemed finished. If there were fewer than 80% free cells, the trypsinization was given a few more minutes to take effect. Trypsinization was limited to a total of 10 minutes to reduce the amount of stress to which the cells were exposed.

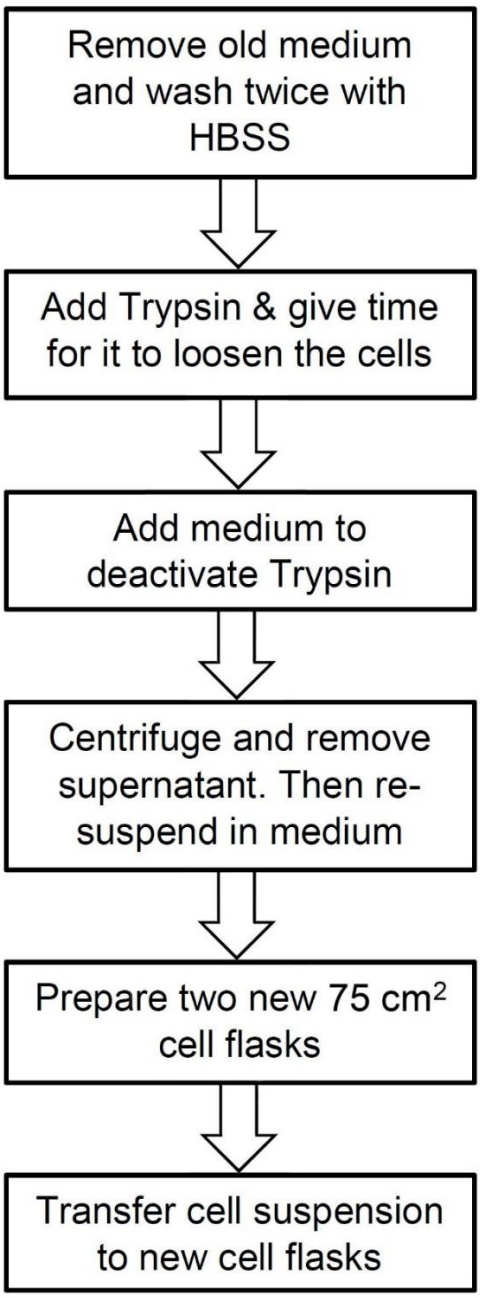

Figure 3. Flow chart for CHSE-214 cell culture splitting. The process involves removing the old medium from the cell flask and washing the flask twice with HBSS. There is then added Trypsin and some time is given to let the cells loosen from the substrate. Medium is then added to stop the trypsinization and the cells transferred to a tube. This tube is then centrifuged, the supernatant removed, and the cells are then resuspended in new medium. Two new flasks are then prepared and half of the cell suspension from the tube added to each of the new flasks.

When ready, the flask was placed back in the LAF cabinet and 4.0 mL of medium (referred to in Appendix A1) was introduced to the flask. This inactivated the Trypsin. The flask was tilted from side to side a few times to ensure that the medium came into contact with the entire growing surface of the flask. The content of the flask was then transferred into a 50.0 mL Falcon tube and a cap screwed on to ensure the sterility of the cell suspension. The Falcon tube was then removed from the LAF cabinet and placed in a centrifuge set at 1200 rpm for 3 minutes at speed. After the centrifuge had stopped running the tube was checked to ensure the formation of a cell pellet at the bottom of the tube, otherwise the Falcon tube was placed back for a second round of centrifugation.

In the LAF cabinet once again, the cell pellet was resuspended in 1.0 mL of medium by pipetting the content up and down several times until there were no visible clusters of cells. A further 5.0 mL of medium was then introduced, and the content of the Falcon tube mixed to ensure an even distribution of cells in the suspension. Then, 3.0 mL of cell suspension was pipetted into each of the new flasks. The content of the new cell flasks was mixed by gently tipping each flask back and forth a few times. Finally, the new flasks were placed back in the incubator set at 20°C.

If the cells were only to be split into a new flask, only one new flask was prepared, to which 17.0 mL of medium was added. To this flask, 3.0 mL of cell suspension was added and the remaining 3.0 mL in the tube was discarded.

When preparing samples for analysis in the flow cytometer the cells were transferred from a 75 cm^2 cell flask to a 6-well 9.6 cm^2 microplate. This method deviated from the regular cell splitting method (referred to in Appendix A2) in that instead of preparing two new 75 cm^2 cell flask a 6-well 9.6 cm^2 microplate was prepared, with 1.0 mL of medium added to each well. The method also differed in that 1.0 mL of cell suspension was transferred from the Falcon tube to each of the 6 wells on the plate instead of 3.0 mL of cell suspension into the two new 5 cm^2 cell flasks.

The cell splitting methods were later altered by changing the amount of time that the Falcon tube was centrifuged for 3–5 minutes (explained in detail in Appendixes A3 and A6). The method was later changed again (explained in Appendixes A4 and A7) by changing the amount of time that the Falcon tube was centrifuged to 5–6 minutes and by using 15.0 mL Falcon tube instead of a 50.0 mL Falcon tube. The amount of time used on the trypsinization process was reduced from 5–10 minutes to 1–2 minutes, and instead of gently tapping the side of the flask, the flask was gently slapped 3–5 times.

2.3 Assessing CHSE-214 cell growth density

Assessment of the density of CHSE-214 cell growth was performed on samples from both the 75cm^2 cell culture flasks and the 6-well 9.6 cm^2 microplate (Appendix A8). The confluency of the cells was measured and noted down. The old medium in the flask was removed and the flask rinsed twice with 5.0 mL of HBSS to remove all traced of the old medium to avoid ay risk of interference in later steps. Then, 1.0 mL of Trypsin was added to loosen the cells from the substrate. The flask was then removed from the LAF cabinet so that it can again be studied under the inverted microscope. When at least 80% of the cells had loosened, the trypsinization was deemed finished. If there were fewer than 80% free cells, the trypsinization was given up to 10 minutes additional time before continuing to the next step. Then, 5.0 mL of medium was added to the flask and the content was mixed by tilting the flask from side to side. Thereafter, 10.0 µL was pipetted from the cell suspension into each chamber of a Bürker counting chamber, and the chamber placed under a microscope. All chambers were counted. The number of cells in the flask was calculated by using the following formula:

$$C = N/(AVF)*1000$$

where C is the cell concentration, N is the number of cells in the chamber, A is the number of squares in the chamber that was counted, and F is the dilution factor.

To assess the density at which CHSE-214 cells grow in each well of a 6-well 9.6 cm^2 microplate, the amount of Trypsin was decreased from 1.0 mL to 0.2 mL and the amount of medium added was increased from 5.0 mL to 0.8 mL (Appendix A9).

2.4 Thermo Fisher flow cytometer protocols for cell cycle analysis

The first methods used were the ones provided with the assay kits from Thermo Fischer Scientific (referred to in Appendixes A11 and A12). A DAPI (Invitrogen^{TM}, # D3571) stock solution (referred to in Appendix A10) as made by adding 2.0 mL of deionized water (dH_2O) to the vial that came in the kit and letting the content dissolve in the water. The vial of DAPI stock solution was then be stored in a refrigerator. A general overview of the steps performed in the cell splitting can be seen in figure 4.

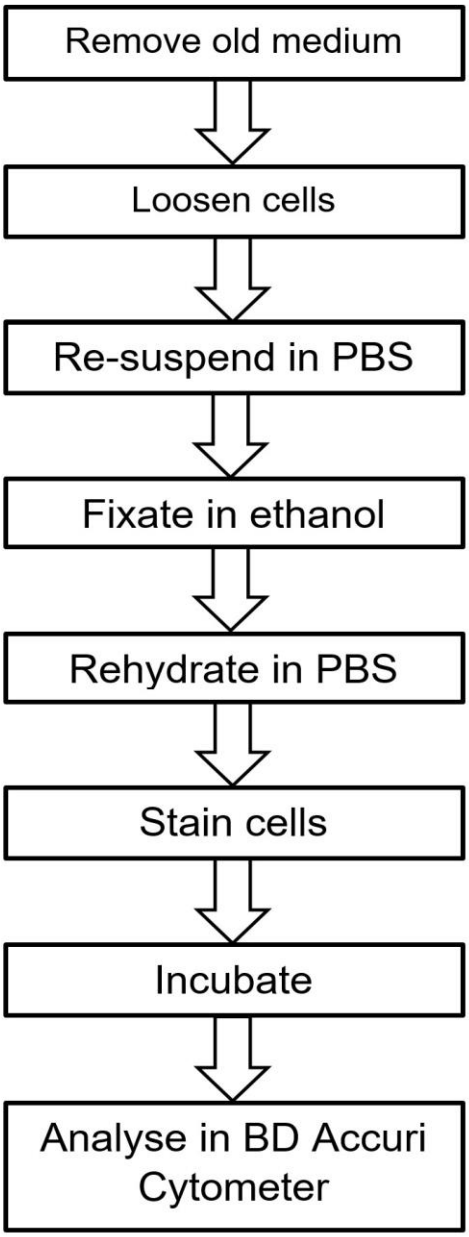

Figure 4. Flow chart for the preparation for CHSE-214 cells for cell cycle analysis with BD Accuri™ Cytometer. The old medium is removed, and the cells are loosened from the substrate according to preferred method. The cells are then centrifuged and re-suspended in PBS. The cells are centrifuged again and fixed for preferred amount of time in ethanol of preferred concentration. The cells are then centrifuged and rehydrated in PBS. The cells are stained according to preferred method and then analysed by flow cytometry.

The first method for sample analysis with DAPI was adapted from the one that method that followed with the Thermo Fisher Scientific DAPI KIT (Appendix A11). To test this method a DAPI staining buffer had to be prepared (A13). To an Erlenmeyer flask there was added 600 mL of MilliQ water. The Erlenmeyer flask was then placed on a magnetic plate and a stirring magnet placed in the flask. The plate was set so that the magnet had a stable rotation. The amount of each substance needed was calculated. 12.112 g of Tris, 8.77 g of NaCl, 0.111 g of $CaCl_2$, and 0.102 g of $MgCl_2$ were weighed and added to the flask. Lastly 1.0 mL of TWEEN® 20 was pipetted into the flask. The content of the flask was given time to dissolve into the solution and was deemed finished when the solution was clear with no visible particles present. A pH electrode was calibrated and placed in the solution. Using 1 molar HCl and 1 M NaOH was used to alter the pH of the solution so that it was 7.4. The solution was then transferred to a 1.0 L volumetric flask and MilliQ water was added to the 1.0 litre mark. The solution was then transferred to a 1.0 L storage bottle with a cork. The bottle was given a description and placed in storage at 4 °C.

There was later made a new DAPI staining buffer solution that swapped out the 1.0 mL of TWEEN® 20 with 1.0 mL of Nonidet P-40 (Appendix A14).

For testing with DAPI a working solution was needed (Appendix A15). This was prepared from the stock solution (Appendix A10). The working solution was made by calculating the total volume needed for the number of samples that were being tested during that session. When the total volume needed was calculated the correct amount of DAPI stock solution was be pipetted into a 15.0 mL falcon tube containing the corresponding amount of DAPI staining buffer (Appendix A13). The solution was be mixed by pipetting.

When the TWEEN® 20 was swapped out the DAPI stock solution method had to be modified, as explained in Appendix A16. This was done by changing the staining buffer from the one containing TWEEN® 20 to the one containing Nonidet P-40 (Appendixes A13 and A14).

The DAPI protocol provided with the kit is explained in Appendix A11. It involved collecting a cell suspension of 2×10^5 to 1×10^6 cells. The suspension was then to be centrifuged at an unspecified setting before removing the supernatant. The tube was then to be tapped to resuspend the pellet in the residual supernatant that could not be removed. To the tube there was to be added 1.0 mL of PBS at room temperature. The full volume of resuspended cells was then to be transferred to a tube containing 4.0 mL of 96% ethanol at –20 °C by pipetting the cell suspension slowly into the ethanol while vortexing at full speed. The cells ware then to be let to fixate in the ethanol for 5 to 15 minutes. After fixation the cell suspension was then to be centrifuged, again at an unspecified setting before removing the supernatant. The tube was to be tapped and to loosen the pellet and 5.0 mL of PBS at room temperature to be added and the cells allowed 15 minutes to rehydrate. the DAPI stock solution was to be diluted to 3 μM in a staining buffer (100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM CaCl2, 0.5 mM MgCl2, 0.1% Nonidet P-40) explained in Appendix A16. A 1.0 mL volume would be required for each cell sample. The solution was then centrifuged for a final time and the supernatant discarded. The tube was then to be tapped and a 1.0 mL of DAPI working solution to be added to the tube and then given 15 minutes to incubate at room temperature. The sample could then be analysed in the flow cytometer.

The Thermo Fisher PI/RNase protocol provided with the kit is explained in Appendix A12. It involved collecting a sample of 1×10^6 cells. These cells could then be fixed according to a preferred protocol; in the case of this thesis, the method was the same as the one explained in Appendix A11. The cell suspension would be centrifuged at an unspecified setting before removing the supernatant. The tube was then to be tapped to resuspend the pellet in the residual supernatant that could not be removed. To the tube there was to be added 1.0 mL of PBS at room temperature. The full volume of resuspended cells was then to be transferred to a tube containing 4.0 mL of 96% ethanol at –20 °C by pipetting the cell suspension slowly into the ethanol while vortexing at full speed. The cells ware then to be let to fixate in the ethanol for 5 to 15 minutes. After fixation the cell suspension was then to be centrifuged, again at an unspecified setting before removing the supernatant. The tube was to be tapped and to loosen the pellet and 5.0 mL of PBS at room temperature to be added and the cells allowed 15 minutes to rehydrate. To the

tube there was to be added 0.5 mL of FxCycleTM PI/RNase Staining Solution (Invitrogen™, # F10797) stain to each flow cytometry sample, and the sample mixed well. The sample was then to be given 15 to 30 minutes to incubate. The sample could then be analysed in the flow cytometer.

2.5 Cell analysis

After all the cell fixation and staining procedures, the analysis with the flow cytometer could be carried out. First, the flow cytometer had to be calibrated, as explained in Appendix A17. For the calibration five tubes were used with different components. The first two tubes had to be filled with 'cleaning' and 'decontamination' solution from the flow cytometer. Another tube had to be filled up with ultrapure water. Other two tubes had to be filled up with Spherotech 8-Peak Validation Beads (BD Accuri™ Cytometers, #653144) and Spherotech 6-Peak Validation Beads (BD Accuri™ Cytometers, #653145), respectively. The whole procedure of how to calibrate the flow cytometer is explained in Appendix A17.

Once the flow cytometer was calibrated, it was ready to analyse the samples, following the procedures listed in the Appendix A.

2.6 Sample collection and analysis optimisation

The first experiment was stained with DAPI. The cells were split and transferred to a microplate according to the method described in Appendix A5. The cells were then given 24 hours to grow and proliferate at 20 °C. The staining and analysis were performed following Appendix A18. After 24 hours the confluency of the cells was checked using an inverted microscope. The old medium of each well was removed and discarded. Each well was then rinsed twice with 0.5 mL of HBSS. 0.2 mL of Trypsin was then added, and the Trypsin was given time to take effect, that being no more than 10 minutes. The microplate was tapped a few times and studied under the inverted microscope. When at least 80% of the cells had loosened from the substrate 0.8 mL of medium was added to the wells. The content of the wells was mixed by gently tilting the microplate back and forth a few times before the contents of each well was transferred into individual 15 mL Falcon tubes. The tubes were centrifuged for 3 minutes at 1200 rpm at speed.

The tubes were checked for the formation of a pellet before being resuspended by pipetting in 1.0 mL of PBS at room temperature. The full volume of the tubes was then transferred into new 15 mL Falcon tubes containing 4.0 mL of absolute ethanol at −20 °C. This was done by pipetting the cell suspension slowly into the new tubes while they were being vortexed at full speed. The cells were fixated for 15 minutes in the freezer at −20 °C.

After fixation the tubes were centrifuged for 5 minutes at 1200 rpm at speed. The supernatant was then removed and discarded. The tubes were then tapped to loosen the cell pellet and resuspended in 5.0 mL of PBS. After 15 minutes of rehydration in the PBS the tubes were centrifuged for 5 minutes at 1200 rpm at speed. During the rehydration process a DAPI working solution was prepared according to the method described in Appendix A15. The supernatant was removed, and the tubes were tapped to loosen the cell pellets and 1.0 mL of DAPI working solution was added to each tube. The tubes were given 15 minutes to incubate in the dark. The samples were then transferred into sample tubes and analysed in the flow cytometer that had previously been calibrated according to the method described in Appendix A17. The flow cytometer was set 20,000 events at medium speed.

The method was repeated, but differed from Appendix 18 in that step 14, the second centrifugation was changed from 3 minutes to 5 minutes (Appendix A19). Along with DAPI method an adaptation of the Thermo Fischer method for PI/RNase was tested (Appendixes A12 and A20).

For making samples to be analysed with PI/RNase cells that were used were collected according to the method described in Appendix A6. The old medium of each well was removed and discarded. Each well was then rinsed twice with 0.5 mL of HBSS. 0.2 mL of Trypsin was then added, and the Trypsin was given time to take effect, that being no more than 10 minutes. The microplate was tapped a few times and studied under the inverted microscope. When at least 80% of the cells had loosened from the substrate 0.8 mL of medium was added to the wells. The content of the wells was mixed by gently tilting the microplate back and forth a few times before

the contents of each well was transferred into individual 15 mL Falcon tubes. The tubes were centrifuged for 3 minutes at 1200 rpm at speed. The tubes were checked for the formation of a pellet before being resuspended by pipetting in 1.0 mL of 1 × PBS at room temperature. The full volume of the tubes was then transferred into new 15 mL Falcon tubes containing 4.0 mL of absolute ethanol at −20 °C. This was done by pipetting the cell suspension slowly into the new tubes while they were being vortexed at full speed. The cells were fixated for 15 minutes in the freezer at −20 °C.

After fixation the tubes were centrifuged for 5 minutes at 1200 rpm at speed. The supernatant was then removed and discarded. The tubes were then tapped to loosen the cell pellet and resuspended in 5.0 mL of 1 × PBS. After 15 minutes of rehydration in the PBS the tubes were centrifuged for 5 minutes at 1200 rpm at speed. The supernatant was removed, and the tubes were tapped to loosen the cell pellets and 0.5 mL of PI/RNase was added to each tube. The tubes were given 30 minutes to incubate in the dark. The samples were then transferred into sample tubes and analysed in the flow cytometer that had previously been calibrated according to the method described in Appendix A17. The flow cytometer was set at 20,000 events at medium speed.

After replacing the TWEEN® 20 buffer solution with the Nonidet P-40 buffer solution the method used in Appendix 19 was swapped out for the method in Appendix A21. The difference being that the DAPI working solution was prepared according to the method described in Appendix A16 rather than according to the method described in Appendix A15. With the new changes to the DAPI protocol there was also tried a new method, as explained in Appendix A22. This method was a fusion of the methods in Appendixes A20 and A21, the samples were treated with both DAPI and PI/RNase. It differed from the method in Appendix 21 in that there was added 0.5 mL of DAPI working solution instead of 1.0 mL and that there was added 0.5 mL of PI/RNase. The incubation time was also extended from 15 minutes to 30 minutes. The cells that were used were collected according to the method described in Appendix A6.

The amount of time that the cells were centrifuged at in step 9 for the methods in Appendixes A20, A21, and A22 was changed from 5 minutes to 6 minutes as explained in Appendixes A23, A24, and A25. The cells that were used were collected according to the method described in Appendix 6. Another noticeable change to the methods of Appendixes A23, A24, and A25 from the previous three is that after the confluency check there is was used a cell scraper to loosen cell from the interior of the well. Instead of removing and discarding the old medium it was rather collected and stored in individual 15 mL Falcon tubes. Likewise, the HBSS that was used to rinse the wells was collected and stored in the respective Falcon tubes. After the trypsinization and adding the 0.8 mL of medium, the mixture was transferred to the tubes containing the old medium and HBSS.

A large-scale experiment was carried out where fifteen different methods were tested. These methods were based on the method explained in Appendix A25, and were divided into four different series: A, B, C and D. The cells were split from 75 cm^2 cell culture flasks to 6 well 9.6 cm^2 microplates according to the method explained in Appendix A7. All samples were collected from the microplates according to the same method.

The confluency of the microplates was evaluated under an inverted microscope before the old medium was removed and discarded. The interior of the wells was rinsed twice with 0.5 mL of HBSS, and any residues of HBSS was removed and discarded. Then there was added 0.2 mL of Trypsin. The Trypsin was distributed over all the cells by tilting the microplates back and forth. The trypsinization was given up to 2 minutes before the microplates were gently hit a few times and then studied under an inverted microscope. When at least 80% of the cells had loosened from the interior of the wells the trypsinization was complete. To each well there was then added 0.8 mL of medium. The content of each well was mixed by tilting the microplates back and forth a few times and then transferred to a 15 mL Falcon tube. The Falcon tubes were then centrifuged at 1200 rpm for 6 minutes at speed. The tubes were checked for the formation of a cell pellet before removing the supernatant. The pellets were resuspended in 1.0 mL of 1 × PBS by pipetting before being pipetted into a 15 mL Falcon tube being vortexed at full speed containing 3.0 mL of either absolute ethanol in the case of series A and series B, or 70% ethanol diluted

from absolute in the case of series C and series D. The samples were then fixed by being stored at −20 °C in the freezer for between 15 minutes and 24 hours, depending on the method as explained in the sections below.

The A-series consisted of altering the amount of time that the cells were fixated for, the staining volume of DAPI working solution and PI/RNase that was added to each sample and the amount of time the samples were given to incubate after staining. In the first two methods, Appendixes A26 and A27, the samples were fixed for 15 minutes. The samples were then centrifuged for 5 minutes at 1200 rpm at speed. The supernatant was then removed and discarded, and then rehydrate in 3.0 mL of 1 × PBS for 20 minutes. The tubes were tapped a few times to dissolve the pellet in the PBS. After rehydration the samples were centrifuged for 5 minutes at 1200 rpm at speed. The supernatant was then removed, and the samples were stained with either 0.50 mL of DAPI working solution and 0.50 mL of PI/RNase or with 0.50 mL of DAPI working solution and 0.50 mL of PI/RNase following Appendix A26 and Appendix A27 respectively. The stained samples were then incubated for 30 minutes or 2 hours in the dark following Appendix A26 and Appendix A27 respectively.

The third and fourth methods (Appendix A28 and Appendix A29 respectively) differed from the first two methods in that the amount of time given to the fixation process was changed from 15 minutes to 1 hour. The method explained in Appendix A28 is otherwise identical to the method explained in Appendix A26, and the method explained in Appendix A29 is otherwise identical to the method explained in Appendix A27.

The fifth and sixth methods (Appendix A30 and Appendix A31 respectively) were the same as the methods explained in Appendixes A26 and A27, except that the amount of time given to the fixation process was altered to 2 hours instead of 15 minutes.

The B-series methods differed from the A-series methods in that the fixation time was changed to 24 hours from the 15 minutes, 1 hour, or 2 hours from the A-series. A third incubation time, of 1 hour, was added to the 30 minutes and 2 hours of incubation found in the A series. The method explained in Appendix A32 being stained with 0.50 mL of both DAPI working solution and PI/RNase, and an incubation time of 30 minutes in the dark at room temperature. The method explained in Appendixes A33 and A34 both being stained with 0.25 mL of both DAPI working solution and PI/RNase, and an incubation time of 1 hour and 2 hours respectively.

The C-series methods were identical to the methods in the B series apart from swapping out the absolute ethanol used for fixing the cells with 70% ethanol that was prepared from absolute ethanol, as mentioned earlier. The methods are explained in detail in Appendixes A35, A36, and A37.

The D-series methods were identical to the C-series methods, except that the second centrifugation step was changed from 1200 rpm for 5 minutes at speed to 2000 rpm for 6 minutes at speed. The methods are explained in detail in Appendixes A38, A39, and Appendix A40.

2.7 Optimised protocol

The final method is explained in Appendix A41. The confluency of the cells is evaluated under an inverted microscope before the old medium is removed and discarded. The interior of the well is then rinsed twice with 0.5 mL of HBSS, and any residues of HBSS removed and discarded. Then add 0.2 mL of Trypsin. The Trypsin is distributed over all the cells by tilting the microplate back and forth. The trypsinization is then given up to 2 minutes before the microplate is gently slapped 3–5 times before being studied under an inverted microscope. When at least 80% of the cells have loosened from the interior of the wells the trypsinization is complete. To the well there is then added 0.8 mL of medium. The content of the well is mixed by tilting the microplate back and forth a few times and then transferred to a 15 mL Falcon tube. The Falcon tube is then centrifuged at 1200 rpm for 6 minutes at speed. The tube is then checked for the formation of a cell pellet before removing the supernatant. The pellet is then resuspended in 1.0 mL of 1 × PBS

by pipetting before being pipetted into a 15 mL Falcon tube being vortexed at full speed containing 3.0 mL of absolute ethanol. The sample is then stored at –20°C in the freezer for at least 24 hours to fix.

After fixation the samples are centrifuged for 6 minutes at 2000 rpm at speed. The supernatant is then removed and discarded. Any remaining ethanol was given a minute or two to evaporate from the tube, before being tapped and adding 0.5 mL of PI/RNase to the sample. The suspension is then pipetted trough a strain into a sample tube. The sample is then given 2 hours to incubate in the dark bore analysis in the flow cytometer. The flow cytometer is set to 40,000 events at slow speed.

2.8 Cell cycle stress analysis

For the experiment, twelve 75cm^2 cell culture flasks with a confluence of 70% were split into 24 microplates according to the method explained in Appendix A7. 0.5 mL of cell suspension was transferred into each of the 12 wells. 1.5 mL of medium was then added to each well before placing the lid on the microplates and placing them in the incubators. There were additional microplates prepared as backups. Each flask was split into two microplates where one was labelled 20 °C and the other 26 °C. Each microplate was given a number counting from 1 to 10. This was to make sure that when a microplate incubated at 20 °C was harvested the corresponding microplate incubated at 26 °C was also harvested at the same time. The microplates marked with 20 °C were placed in an incubator set to 20 °C, and the microplates marked with 26 °C were placed in an incubator set to 26 °C.

After 24 hours in the incubator 3 wells from two corresponding microplates one from each incubator were harvested and fixed according to the method explained in Appendix A41. These samples were named time 0 (T0). Harvesting and fixing samples of 3 wells from two corresponding microplates were repeated for 2 hour (T1), 4 hours (T2), 6 hours (T3), 8 hours (T4), 18 hours (T5), 22 hours (T6), 26 hours (T7), 30 hours (T8), 42 hours (T9), 52 hours (T10), and 56 hours (T11).

After the fixated samples had been stored at –20 °C for more 24 hours, they were centrifuged, stained, and analysed according to the method described in Appendix A41.

3. Results

3.1 Cell culture

Throughout the duration of the experiment the cell proliferation continued at an even pace. The duration of time between each split in the cell line varied between two and three days, as was expected of these cells (Figure 5).

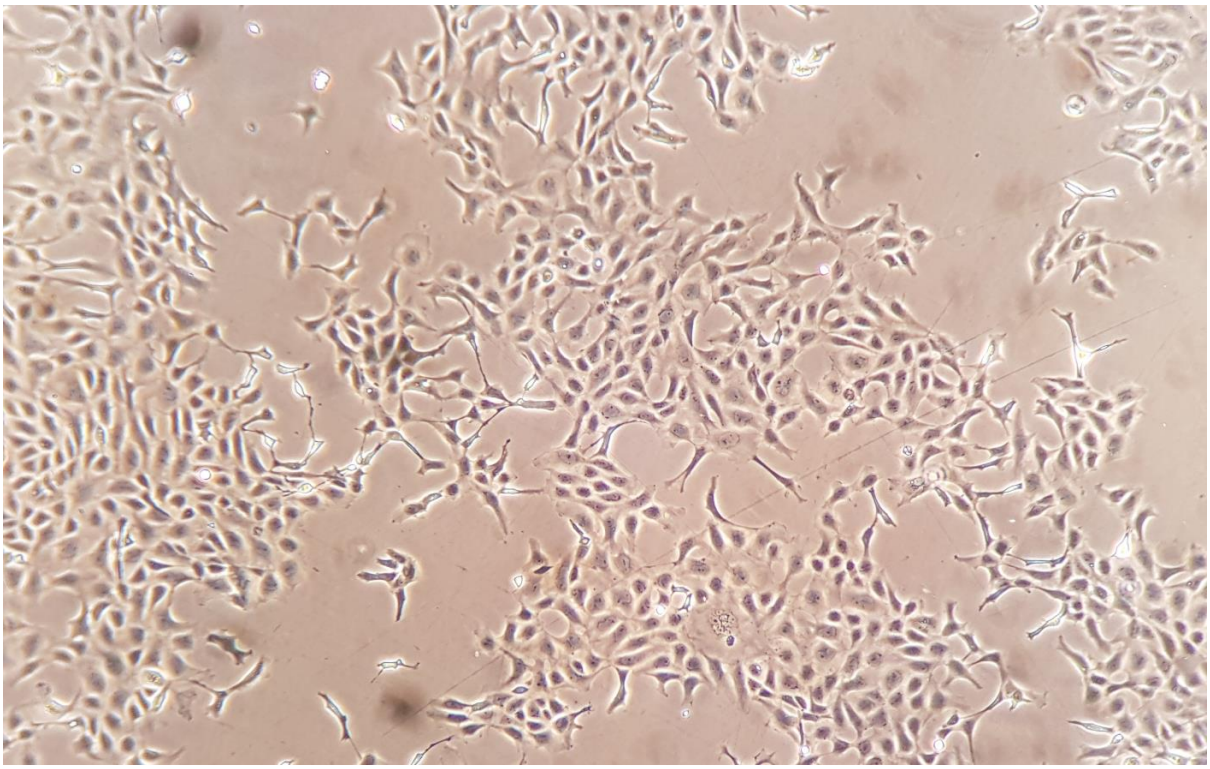

Figure 5. Normal growth of cells in a 75 cm2 cell flask. Image taken under an inverted microscope at 100 X magnification (Motic AE31).

None of the cell flasks or microplates developed any form of contamination. However, it was found that the medium inside the wells of the microplates could at times dry out (Figure 6). Figure 6 shows the wells of the microplate progressively drying out from right to left. This would begin in one corner of the plate and progressively affect the other plates.

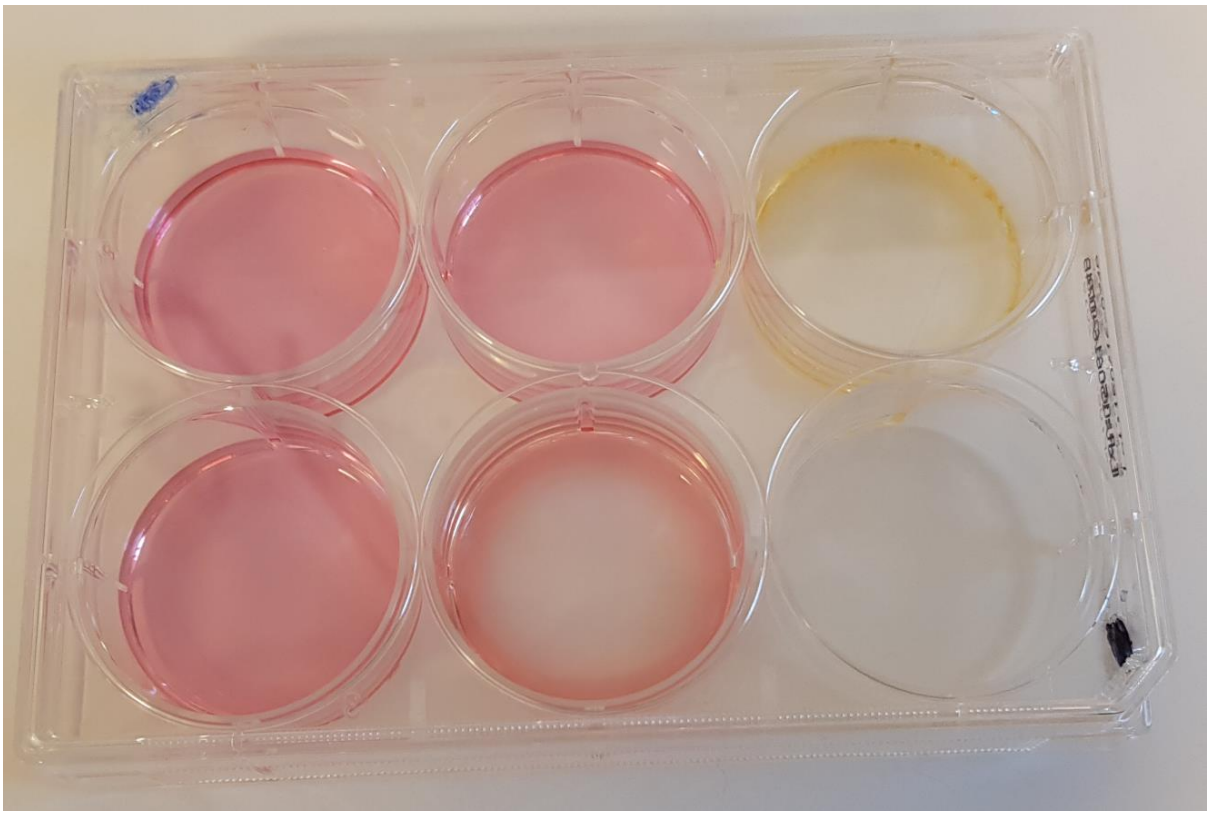

Figure 6. Progressive evaporation of CHSE-214 growth medium (referred to in Appendix A1) from a microplate during incubation. Top left of the microplate shows a well that has not been affected by the evaporation. Bottom right of the microplate shows a well where all of the growth medium has evaporated. Top right of the microplate shows a well where almost all the medium has evaporated, leaving only small amounts of yellowish liquid around the edge.

When this happened there could be found crystalline structures forming inside the wells (Figure 7 and Figure 8). This would eventually kill the cells in the well.

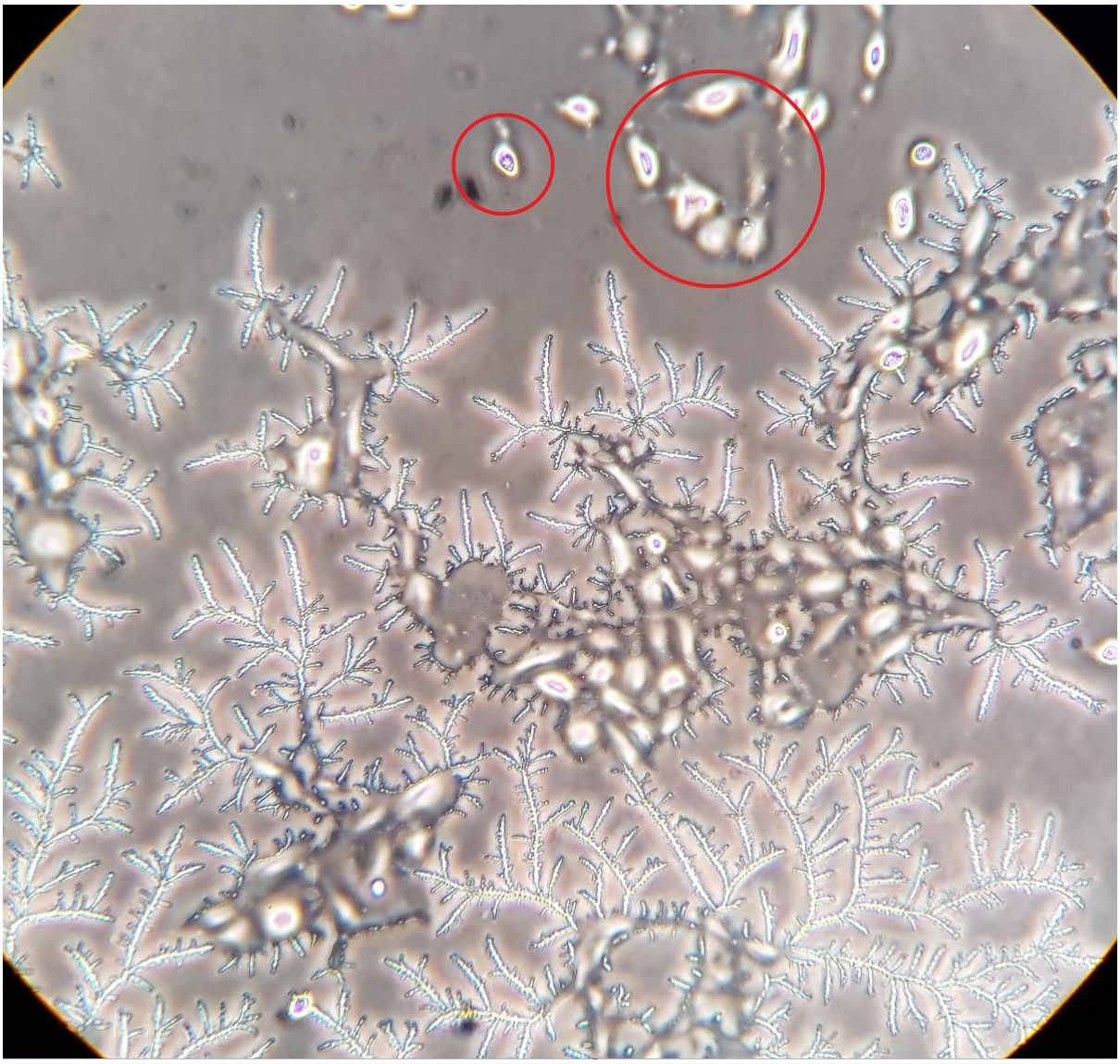

Figure 7. Development of crystalline structures in the wells of a microplate. Dying CHSE-214 cells circled in red. Image taken under an inverted microscope at 100 X magnification (Motic AE31).

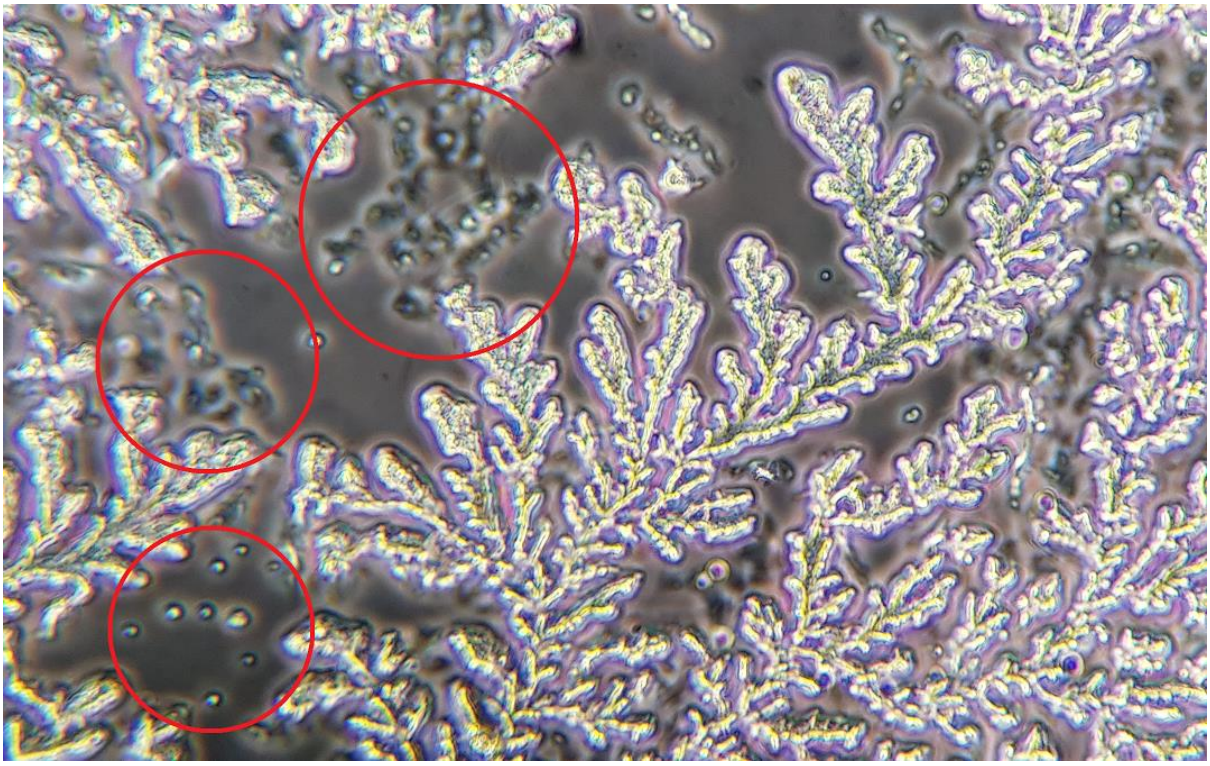

Figure 8. Development of crystalline structures in the wells of a microplate. Dying CHSE-214 cells circled in red. Image taken under an inverted microscope at 100 X magnification (Motic AE31).

3.2 Cell culture splitting

During cell splitting and proliferation it was found that cells that were centrifuged for longer gave higher yields of cells in the new flask compared to cells that were centrifuged for shorter durations. Slapping the side of the cell culture flask 3–5 times after up to 2 minutes of

trypsination gave the best results for free floating cells (Figure 9) and would consistently loosen almost 100% of the cells.

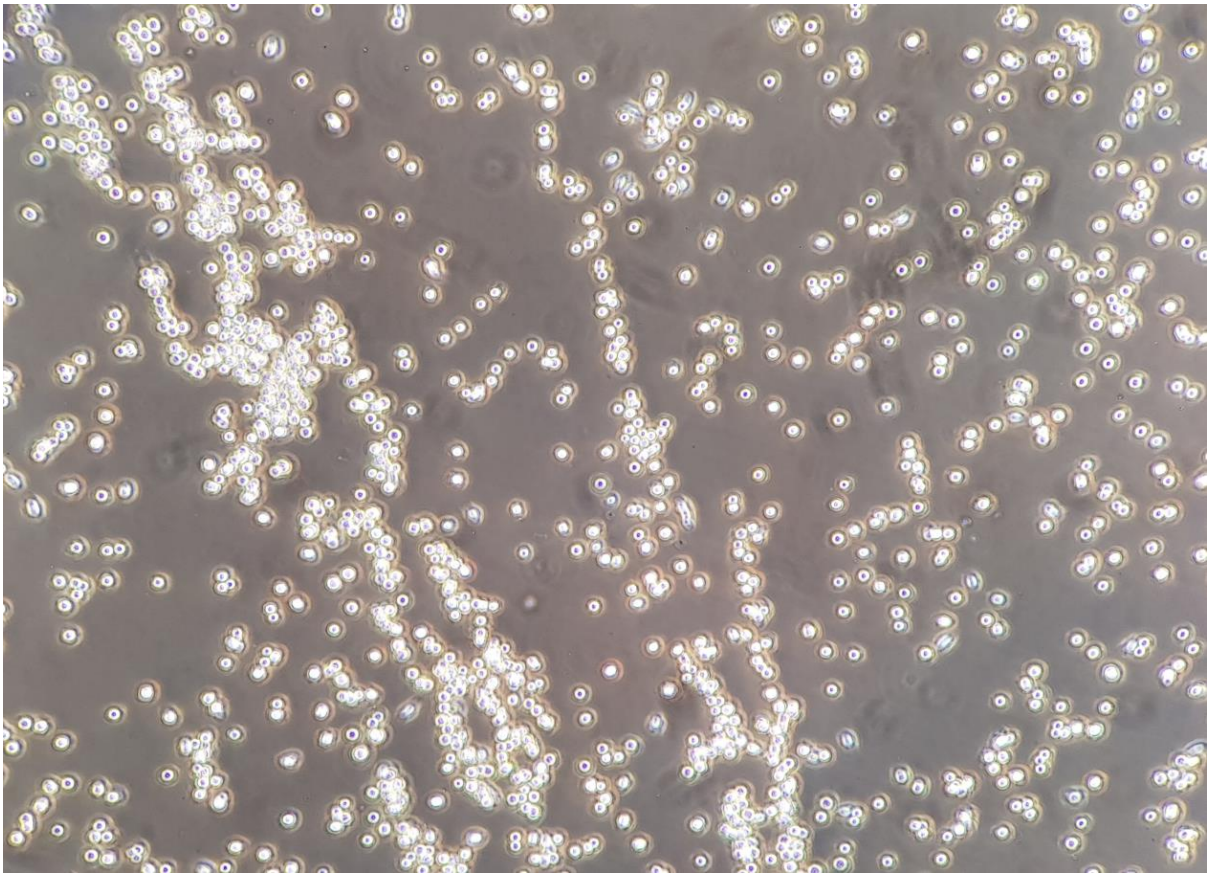

Figure 9. Free floating CHSE-214 cells after trypsinization. The cells can be seen forming two distinct waves as they move through the growth medium referred to in Appendix A1. Image taken under an inverted microscope at 100 X magnification (Motic AE31).

3.3 Cell concentration

The number of cells in the wells of microplates was found to vary from c.230,000 to c.325,000 cells. These were for wells with a confluence between 70% and 90%. In 75cm^2 flasks with a confluence of 70% the number of cells was c.2 million.

3.4 Protocol optimisation

All the results for the protocol optimisation can be found in Appendix B (B1–B49). The different preparation and staining methods had to be tested with the flow cytometer to be able to determine the most suitable method for further sample analysis.

Some of the graphs allow for the comparison of the differences between the forward scatter of light (FSC-A) in the x-axis, and the side scatter of light (SSC-A) in the y-axis, as shown in Figure 10. In this case, it is possible to compare the cell size with the cell's complexity or granularity.

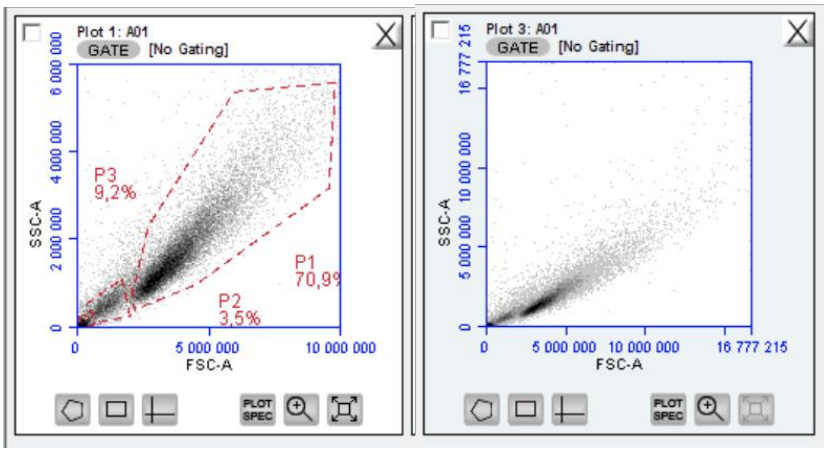

Figure 10. Forward Scatter (FSC-A) vs Side Scatter (SSC-A). Graphic representation showing the relationship between the cell size (FSC-A) and the cell's internal complexity (SSC-A). – Appendix B8.

It is also useful to obtain graphs showing the relationship between FSC-A (A meaning area) and SSC-H (H meaning height)in order to discriminate doublets from single cells, but this was not the main focus on this experiment, so not many graphs of these characteristics were used.

Furthermore, the rest of the graphs showed the relationship between the cell's fluorescence (FL1-A in the case of DAPI and FL2-A in the case of PI) in the x-axis, and the cell number (Count) in the y-axis (Figure 11). This gave us information about the number of cells that had absorbed and

later released specific amounts of energy quantified by several detectors. Depending on the type of cell, or the cell cycle stage in which the cells are found, the fluorescent intensities would be higher or lower.

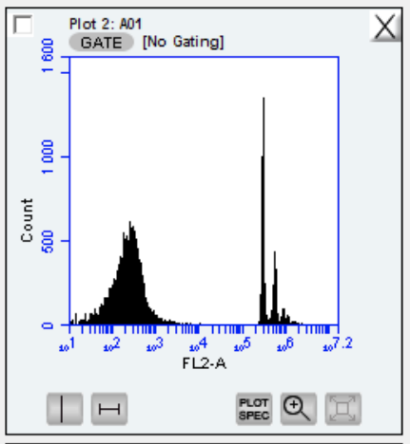

Figure 11. Cell fluorescence (FL2-A) versus cell number (count). Graphic representation showing the relationship between the cell's released energy or fluorescence (FL2-A) and the cell number (count). – Appendix B26

First, the DAPI alone was tested with the flow cytometer (Figure 12). The events were all clustered together in one area and there were no distinct populations of intact DNA or cellular debris/DNA fragments. The DNA size was also very consistent, it was clustered into one peak that leaned to the right of the graph as can be seen in plot 2 in Figure 12.

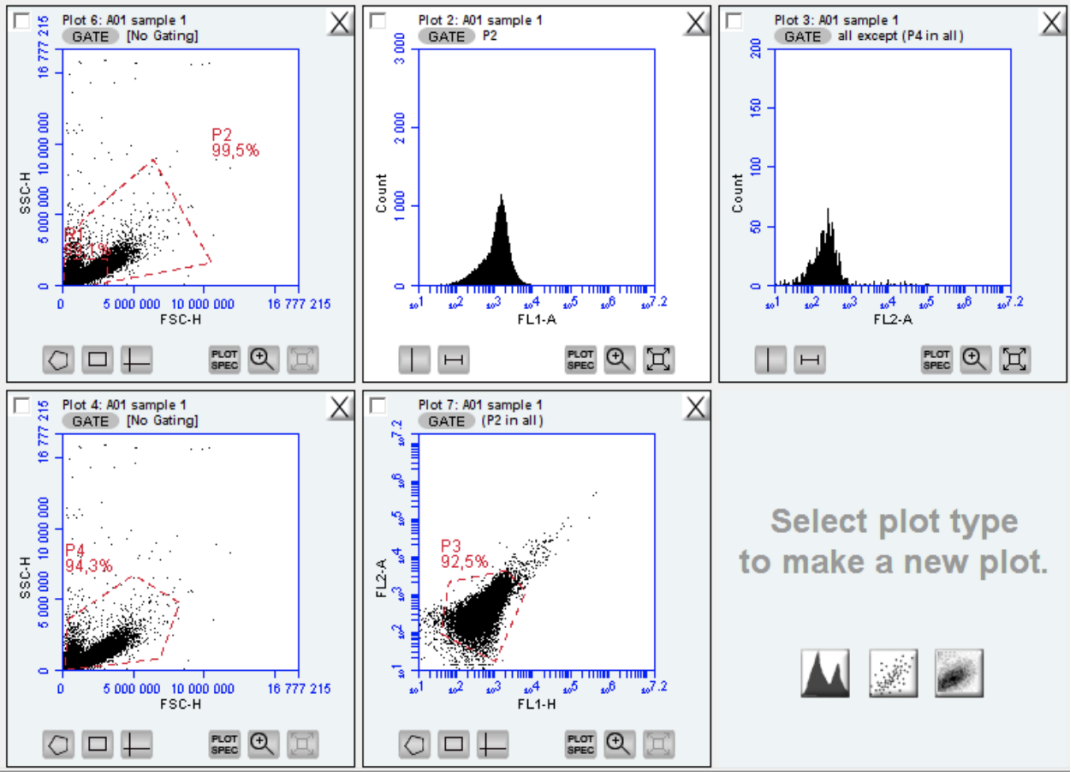

Figure 12. Results from sample stained with DAPI and prepared according to the method described in Appendix A19. – Appendix B4

Both plots 4 and 6 in Figure 12 represent the relationship between the cell size versus the cell's complexity or granularity. In plot 7, the relationship between the cell's different fluorescence according to area and height is shown, so doublets can be discriminated, given the case that two cells pass at the same time through the cytometer.

The samples from early protocols in the optimisation process lacked any distinct populations in the plots showing the amount of DNA and the forward light scatter for DAPI and PI (Figure 13). All the events were grouped together into one large cluster forming a single peak in the plot, as seen in plot 2 in Figure 13.

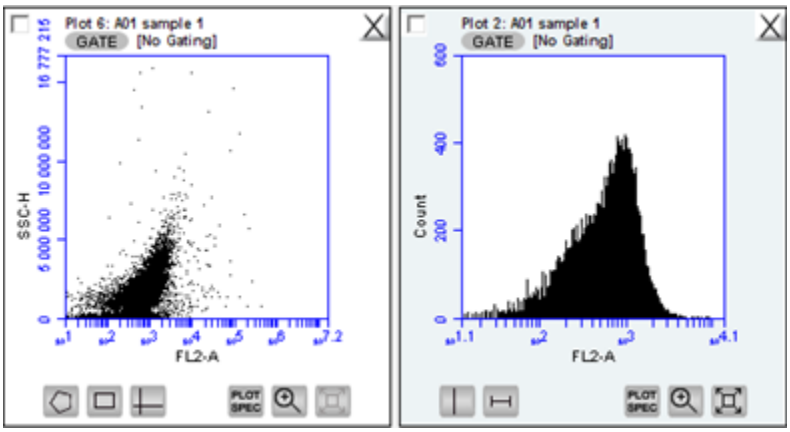

Figure 13. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells grown at 20 °C for 24 hours and stained with DAPI. In plot 2 there can be seen a single peak. Sample prepared according to the method explained in Appendix A18 – Appendix B1

There were no distinct populations of intact DNA or cellular debris/DNA fragments. The DNA size was also very consistent: it was clustered into one peak that leaned to the right of the graph as can be seen in plot 2 in Figure 13. No cell cycles could be observed.

The first samples stained with PI/RNase gave slightly different result compared to the early samples that were stained with DAPI (Figure 13). The sample was prepared according to the protocol explained in Appendix A20 (Figure 14). The graph of the DNA count and size was split into two peaks, the one on the left being very short and the one on the right being very tall (Figure 14). There was not a clear distinction between DNA fragments and intact DNA.

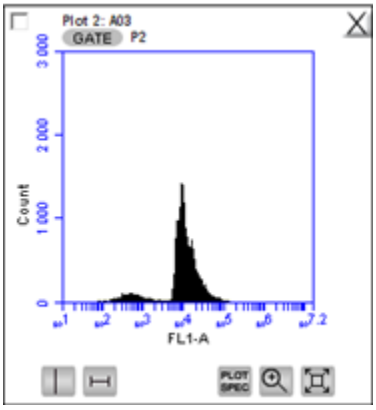

Figure 14. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells stained with DAPI. Plot shows two distinct areas/populations of DNA amount. Sample prepared according to the method explained in Appendix A20. – Appendix B6

Later samples using different protocols for sample preparation gave results were clear populations of intact and fragmented DNA could be seen (Figures 15 and 16). The sample was prepared according to the method described in Appendix A22. The forward light scatter for DAPI gave to sharp peaks, whilst the forward light scatter plot for PI gave a broad and shallow peak, as seen in plot 2 in both Figure 15 and Figure 16. There was no distinct cell cycle visible from the samples. These samples also gave distinct populations on the forward light scatter to side scatter plot as seen in plot 1 in Figure 15. P1 is the intact DNA, and P2 and P3 are clusters of fragmented DNA.

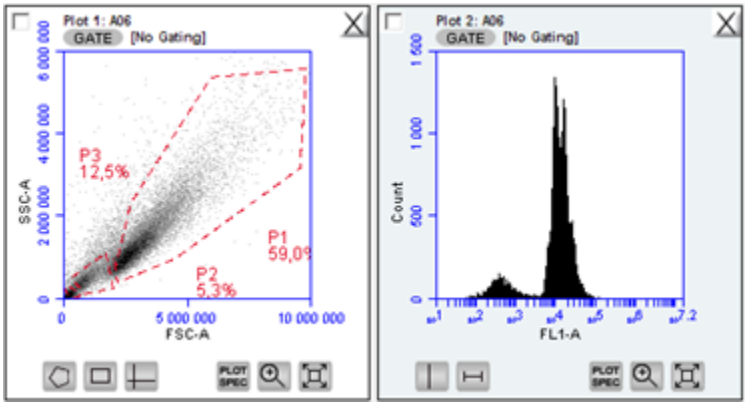

Figure 15. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells grown at 20 °C for 24 hours and stained with DAPI. Sample prepared according to the method explained in Appendix A22. In plot 1 three distinct groupings of events can be seen. P1 indicating whole cells, and P2 and P3 indicating fragmented cells/cellular debris. Plot 2 shows the results of the forward light scatter results for DAPI. – Appendix B14

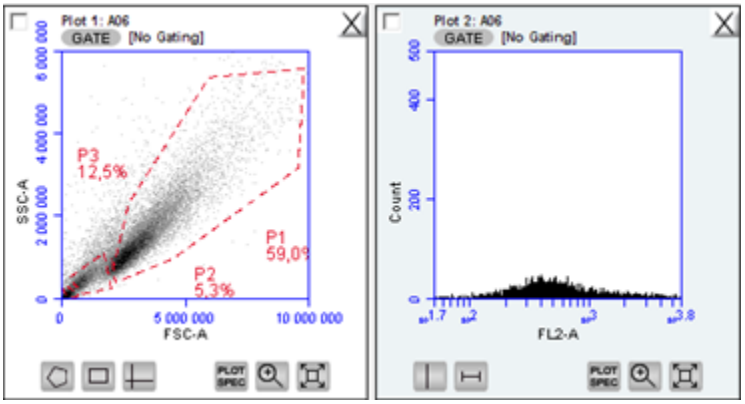

Figure 16. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells grown at 20 °C for 24 hours and stained with PI/RNase. Plot 1 shows the same cells as in Figure 16. Plot 2 shows the results of the forward light scatter results for PI Appendix B15

The cell cycle was first seen when preparing cells according to the protocol explained in Appendix A24 (Figure 17). In this sample, both the cell cycle and the cell debris/fragmented DNA can easily be identified in plot 2, there being two distinct clusters of events on the plot. The clusters seen on the far left of plot 2 indicate fragmented cells/cellular debris and the cluster on the right the cell cycle. It can be distinguished by being a cluster of events with two large peaks

with a flat connecting them. Plot 8 is an amplification of the cell cycle seen in the right half of plot 2.

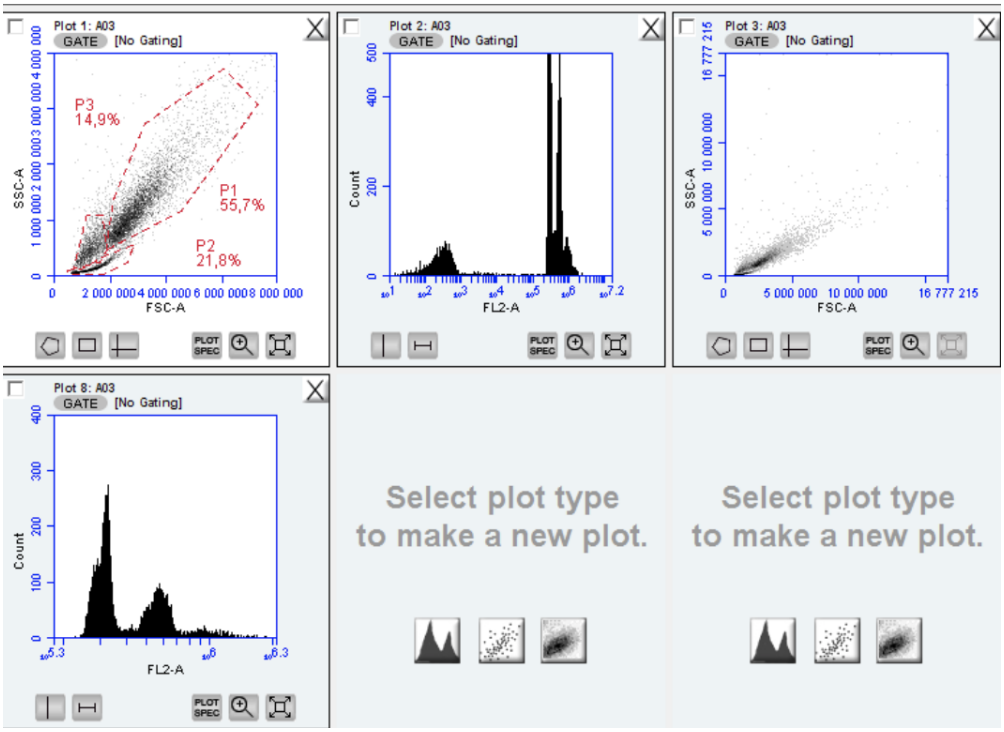

Figure 17. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells grown at 20 °C for 24 hours and stained with PI/RNase. In plot 1 three distinct groupings of events can be seen. P1 indicating whole cells, and P2 and P3 indicating fragmented cells/cellular debris. The cell cycle can we seen on the right side of plot 2. Fragmented cells/cellular debris can be seen on the left side of the same plot. Plot 8 represents an enhanced view of the cell cycle from plot 2. Results from sample stained with PI/RNase and prepared according to the method described in Appendix A24. – Appendix B18

The results obtained from the PI staining were a key point when deciding the definitive optimisation method. When the later samples were stained with just PI/RNase or with both DAPI and PI/RNase, the plots showing the fluorescence given off by the PI gave results where there were clear cell cycles as well as fragmented DNA (Figure 18). This could not be said for the results from DAPI. There were no clear cell cycles, and samples consistently gave first a shallow peak and then a tall peak in the plots, as can be seen in Figure 14, plot 2 in Figure 15, and plot 9 in Figure 18.

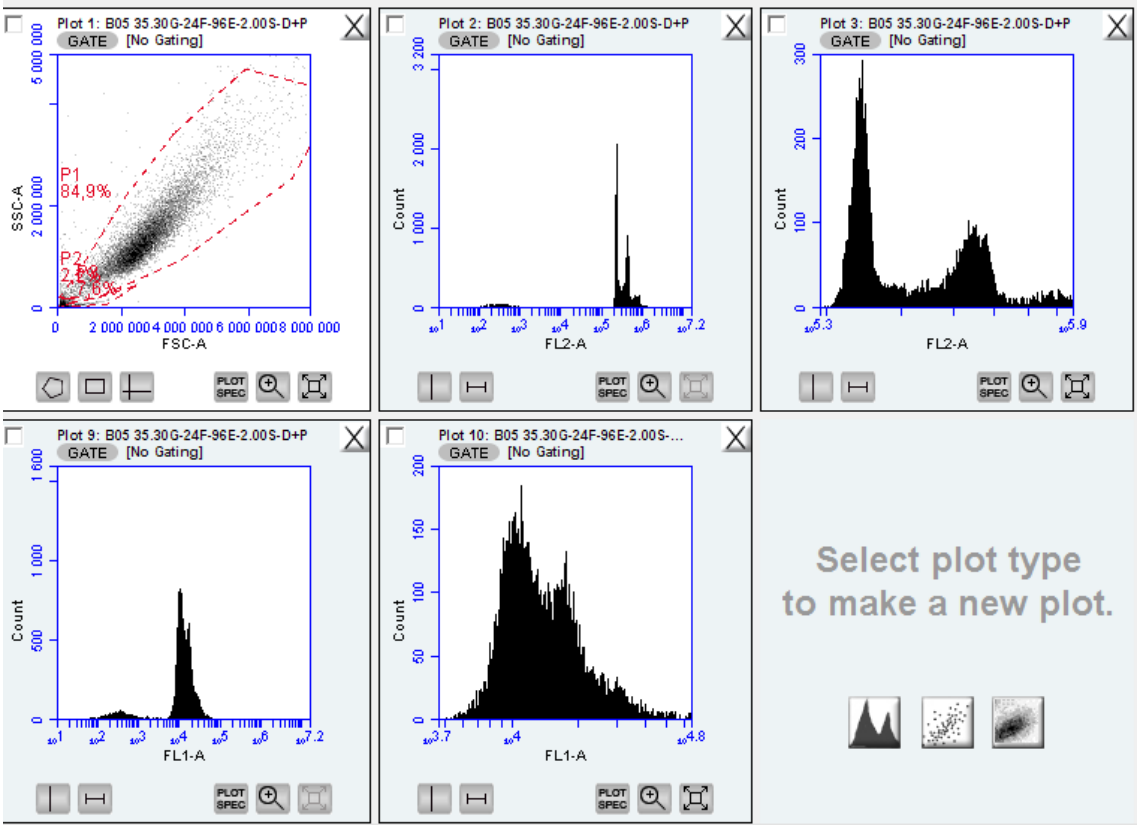

Figure 18. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells grown at 20 °C for 24 hours and stained with both DAPI and PI/RNase. In plot 1 three distinct groupings of events can be seen. P1 indicating whole cells, and P2 and P3 indicating fragmented cells/cellular debris. The cell cycle can we seen on the right side of plot 2. Plot 2 and 3 shows the forward scatter results from PI and plot 9 and 10 show the forward scatter results from DAPI. Fragmented cells/cellular debris can be seen on the left side of the same plot. Plot 3 represents an enhanced view of the cell cycle from plot 2, and plot 10 represents an enhanced view of the peak on the right side of plot 9. There can be seen no distinct cell cycle for DAPI. – Appendix B36.

3.5 Cell cycle analysis

The use of PI/RNase to stain samples was found best suited for the analysis of the cell cycle of CHSE-214 cells (for all results see Appendix C (C1–C57) and Appendix D (D1–D59).

The first control sample, exposed for 0 hours in an incubator set to 20 °C gave clear populations of intact cells and cellular debris, as can be seen in plot 1 in Figure 19. P1 accounting for 85.5%

of all the events. In plot 7, the fragmented DNA marked M3 and the cell cycle are clearly visible, plot 2 showing just the area of plot 7 that contains the cell cycle.

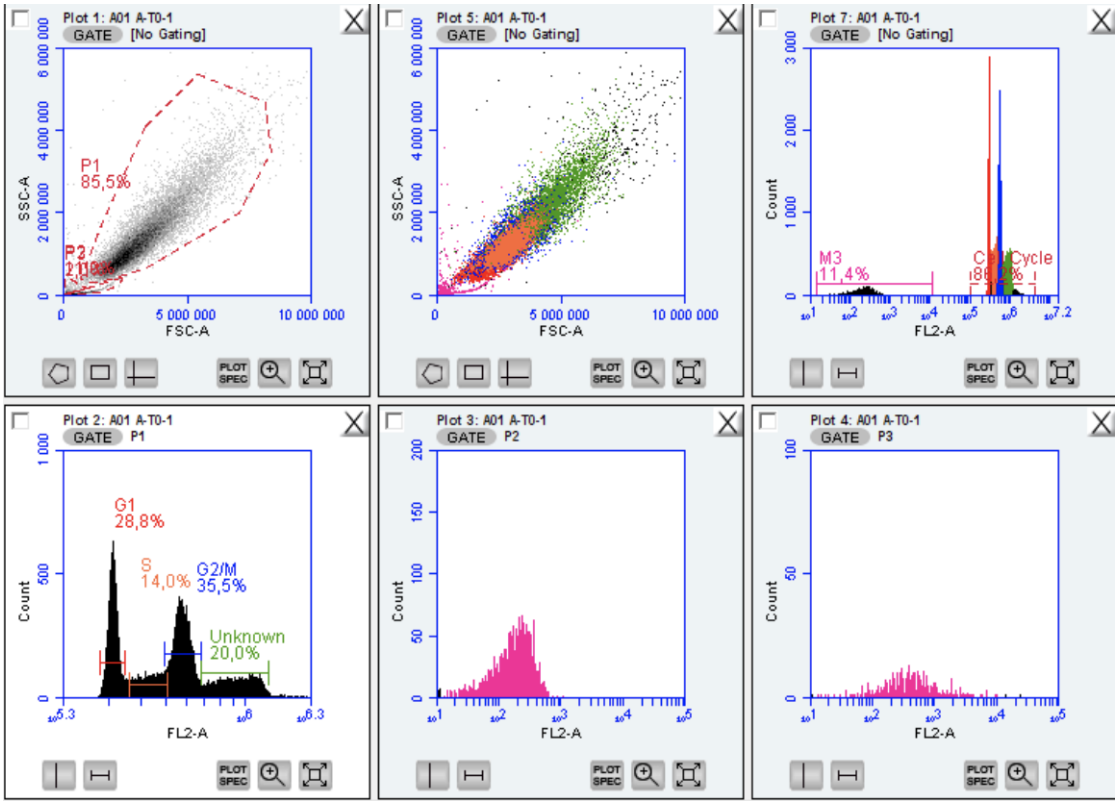

Figure 19. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells grown at 20 °C for 24 hours and stained with PI/RNase. In plot 1 three distinct groupings of events can be seen. P1 indicating whole cells, and P2 and P3 indicating fragmented cells/cellular debris. The cell cycle can we seen on the right side of plot 7. Plot 2 represents an enhanced view of the cell cycle from plot 7. The peaks of the cell cycle phases are named according to the phase the peak represents. – Appendix C1.

The cell cycle is divided into three distinct areas named after the phase of the cells cycle the DNA is in. That being G1, S, and G2/M, there is also marked a fourth area, unknown, this is explained in the discussion. The distinct areas of plot 2 are coloured to distinguish them better, this also allows the different phases of the cell cycle to be seen in plot 5. The highest amount of DNA can be found in the G2/M phase of the cell cycle, accounting for 35.5% of P1. The G1 phase and S phase accounting for 28.8% and 14% respectively of the same plot.

Similar results can be seen from the stress cells with 0 hours of exposure in an incubator set to 26 °C (Figure 20).

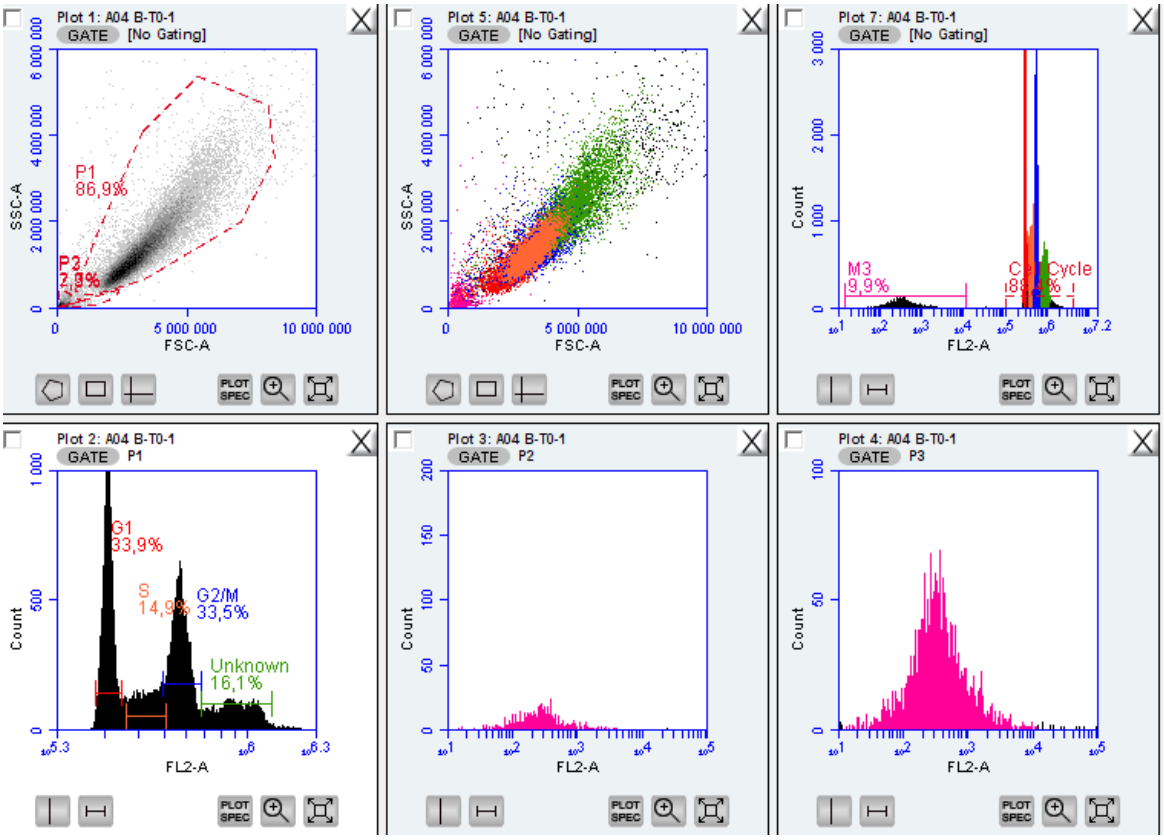

Figure 20. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells grown at 20 °C for 24 hours and stained with PI/RNase. In plot 1 three distinct groupings of events can be seen. P1 indicating whole cells, and P2 and P3 indicating fragmented cells/cellular debris. The cell cycle can we seen on the right side of plot 7. Plot 2 represents an enhanced view of the cell cycle from plot 7. The peaks of the cell cycle phases are named according to the phase the peak represents. – Appendix C4.

Comparing the results from 0 hours of exposure to 26 °C and 26 hours of exposure at 26 °C yields some interesting results (Figure 21).

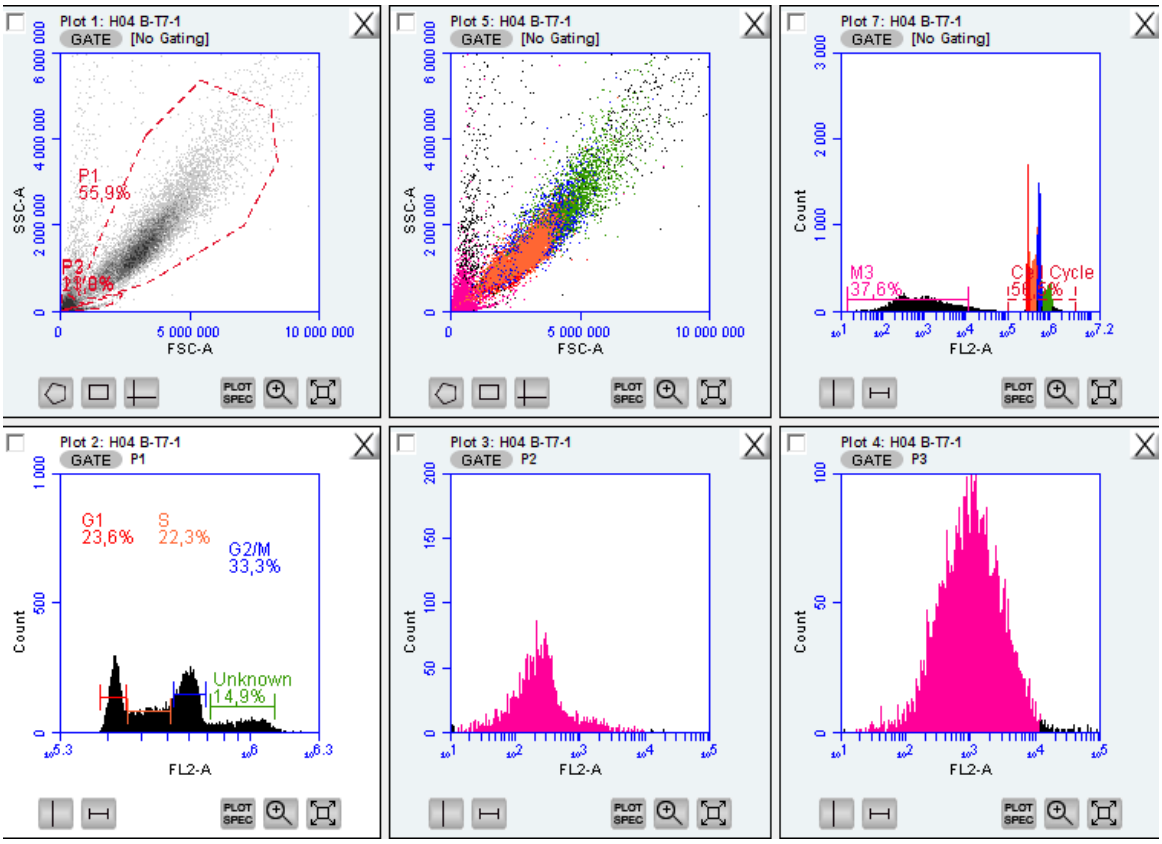

Figure 21. Results from BD Accuri Cytometer showing the forward light scatter to count plots of CHSE-214 cells grown at 20 °C for 24 hours and stained with PI/RNase. In plot 1 three distinct groupings of events can be seen. P1 indicating whole cells, and P2 and P3 indicating fragmented cells/cellular debris. The cell cycle can we seen on the right side of plot 7. Plot 2 represents an enhanced view of the cell cycle from plot 7. The peaks of the cell cycle phases are named according to the phase the peak represents. – Appendix C31.

In plot 7, it can be seen that the cell debris percentage (M3) for the 0 hour of exposure to 26 °C sample was of 9.9%. A big difference can be seen in the case of the cells exposed for 26 hours, as 37.6% of cell debris was found. A similar big difference can be seen in plot 4.

When focusing in plot 2 for each sample, the different stages of the cell cycle can easily be analysed and compared. A comparison of the samples in Figure 18 and Figure 19 shows that the cell count for the G1 phase decreases from a starting 33.9% to a final 23.6%. The same happens for the G2/M phase, where the cell count decreased slightly from 35.5% to 33.3% after exposing

the cells for 26 hours to 26°C. On the other hand, it was found that the number of cells in the S phase increased after being stressed 26 hours at 26°, increasing from a 14% to a 22.3%. Additionally, graphs were created in order to see the results in a more visual and easier way in order to facilitate comparisons of the results.

In Figure 22 the number of cells (%) in the G1 phase is represented for both the control and the stressed cells in different time periods. From the graph is easy to see that most of the time the number of cells at 20 °C is higher than at 26 °C, especially after 26 hours of exposure, where the difference is very noticeable. There are some points along the 56 hours where a greater number of cells were present in the stressed cells (26 °C) than in the control cells (20 °C). These points were after 2, 6 and 42 hours of exposure.

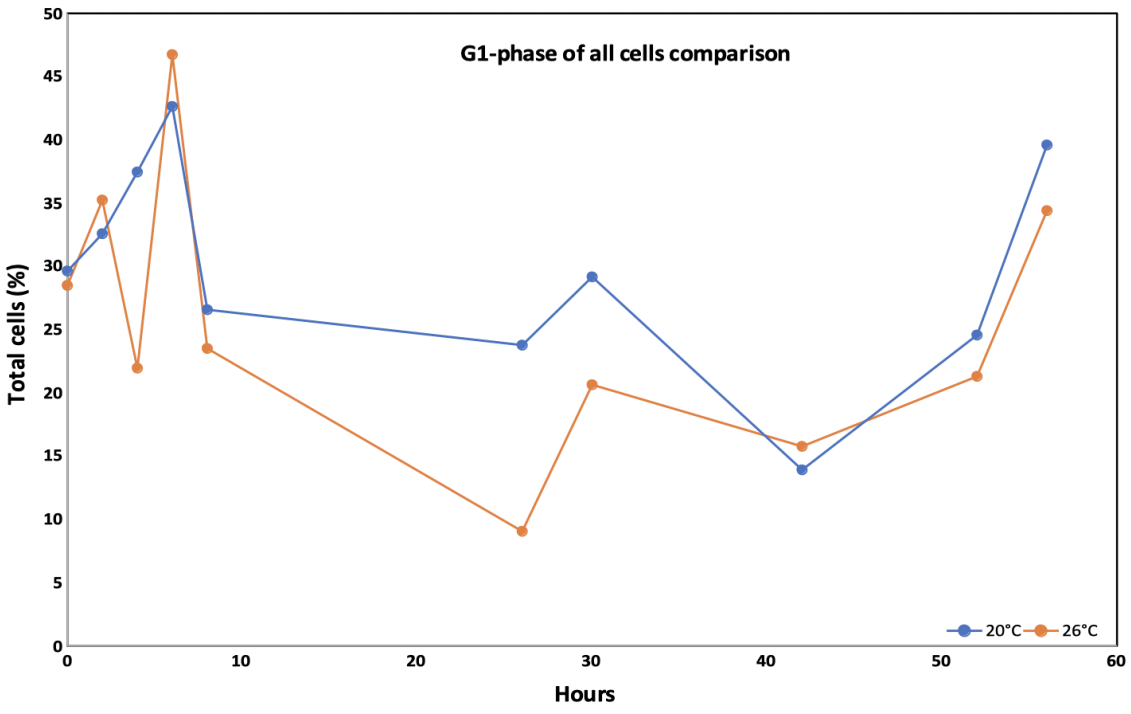

Figure 22. Comparison of the G1 phase at 20 °C and 26 °C. Graphic representation showing the relationship between the exposure time (hours) and temperature (20 °C and 26 °C), and the total cell number as a percentage (including cell debris) in the G1 phase. – Appendix D41

It can also be seen that towards the end of the experiment, both control and stressed cells increase in quantity in a relatively short amount of time.

Similarities can be found at the S phase too (Figure 23). After 2 hours and 26 hours the number of cells is higher when stressed at 26 °C than is the number of the control cells (20 °C). A similar graph pattern is followed as shown in Figure 22, as when 30 hours pass, the number of cells suddenly increases, and a sudden increase towards the end of the experiment can be noticed.

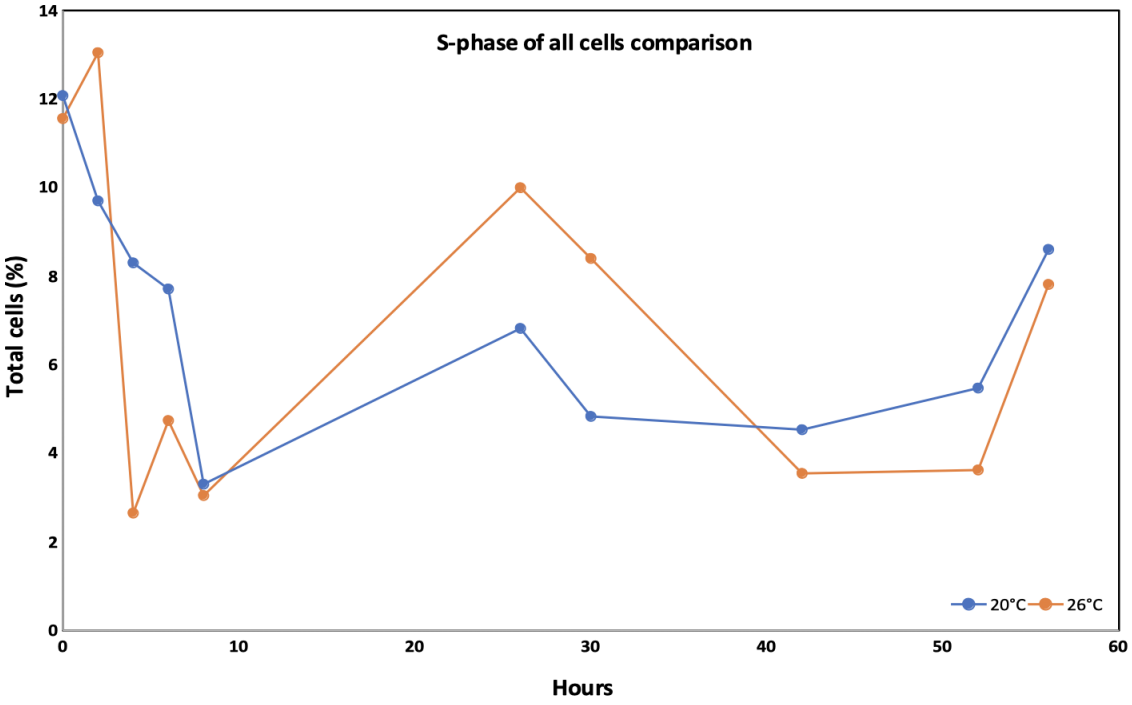

Figure 23. Comparison of the S phase at 20 °C and 26 °C. Graphic representation showing the relationship between the exposure time (hours) to temperature (20 °C and 26 °C) and the total cell number as a percentage (including cell debris) in the S phase. – Appendix D44

In Figure 23 the cell number pattern in the S phase is not that like the G2/M phase (Figure 24).

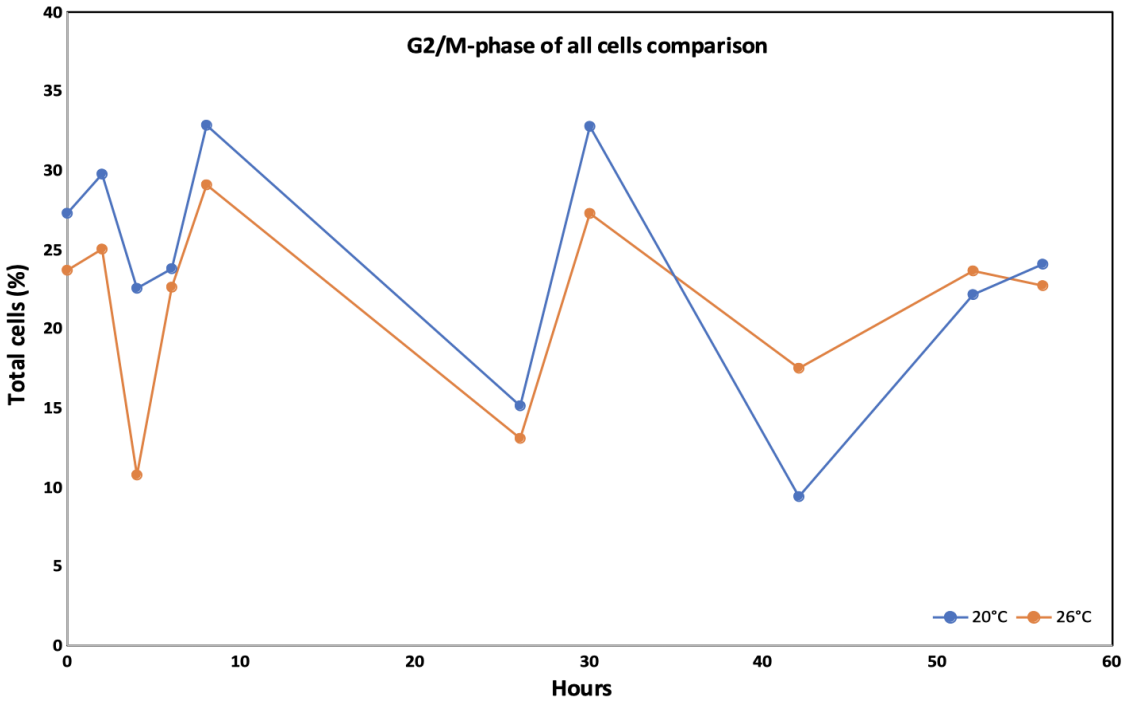

Figure 24. Comparison of the G2/M phase at 20 °C and 26 °C. Graphic representation showing the relationship between the exposure time (hours) to temperature (20 °C and 26 °C) and the total cell numbers as a percentage (including cell debris) in the G2/M phase. – Appendix D47

On the other hand, the cell number pattern followed by both control and stressed cells is quite similar, following the same increasing and decreasing tendencies, with the stressed cells (26 °C) consistently being lower on the graph until the 42 hour mark where the 2 graphs begin to line up at the same level. It can also be observed that the percentage number of stressed cells exceeds that of the control cells when 42 and 52 hours pass.

The percentage number of cell debris could also be compared between the stressed and the control cells. The amount of cell debris in the stressed cells is normally higher than in the control cells, and it can be observed as the experiment is carried out along the 56 hours (Figure 25).

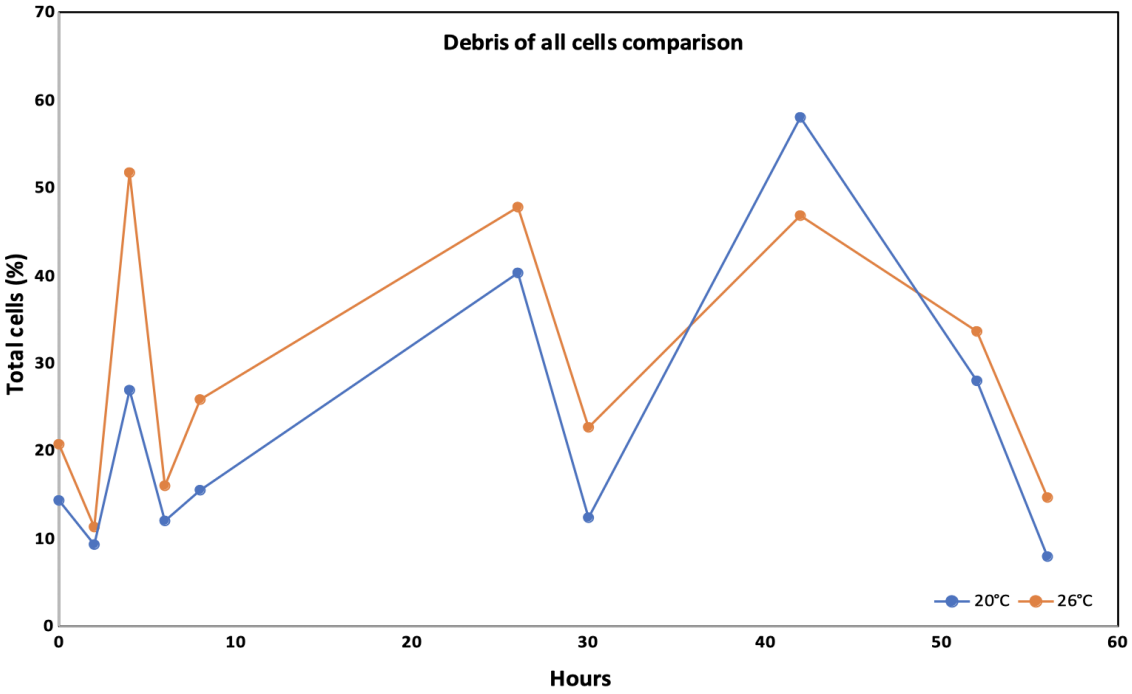

Figure 25. Comparison of cell debris at 20 °C and 26 °C. Graphic representation showing the relationship between the exposure time (hours) to temperature (20 °C and 26 °C) and the total cell number as a percentage of cell debris. – Appendix D50

It can also be observed that after 42 hours, the cell debris percentage of the control cells suddenly exceeded that same percentage of the stressed cells, but ended up lowering towards the end of the experiment.

Not many variations can be seen in the control cells (20 °C) over the 56 hours (Figure 26). A great increase of cell debris can be observed after 42 hours, while the G1, S, and G2/M phases do not show very drastic variations.

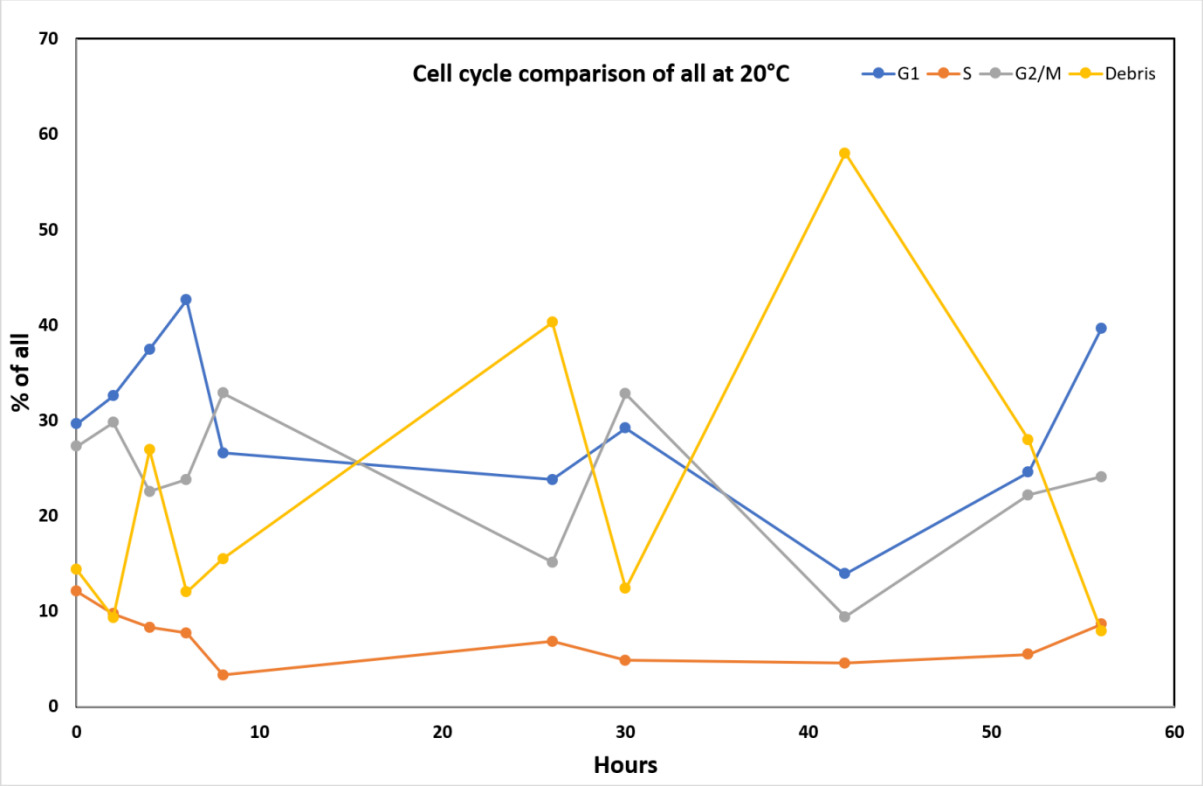

Figure 26. Cell cycle phases for control cells (20 °C). Graphic representation showing the relationship between the exposure time (hours) to temperature (20 °C) and the total cell number as a percentage of cells in the G1, S and G2/M phases. – Appendix D57.

When looking at the stressed cells (26 °C) both the G1 phase and the G2/M phase follow similar variations (Figure 25), just like in the control cells (Figure 24). The cell debris variations are also like those in the control cells, but higher percentages can be observed. The S phase varies very little comparing the control and the stressed cells.

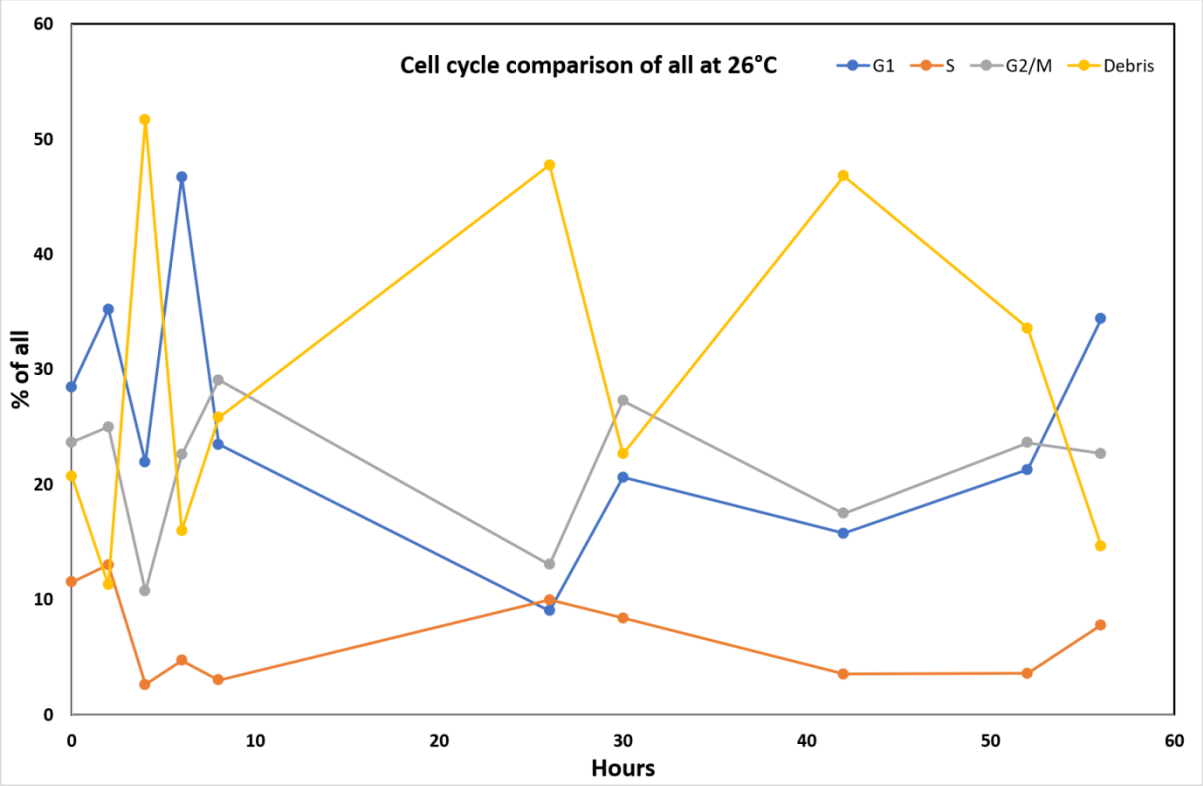

Figure 27. Cell cycle phases for stressed cells (26 °C). Graphic representation showing the relationship between the exposure time (hours) to temperature (26 °C) and the total cell number as a percentage of cells in the G1, S and G2/M phases. – Appendix D59

Moreover, the cell debris percentage seems to drastically decrease in both control and stressed cells after 30 hours of exposure, with 12% cell debris in control cells and 23% cell debris in stressed cells. It can also be pointed out that after 42 hours, the cell debris in control cells was of 58%, higher than stressed cells, with a 47%.

4 Discussion

4.1 Cell culture and cell growth density

Due to the results for the growth density of between 230,000 and 325,000 cell per well at 70% and 90% confluence respectively, it was unclear whether it would be possible to obtain any results from the flow cytometer using the protocols provided by the Thermo Fisher Scientific kits (Appendix 11 and Appendix 12), as they called for between 2×10^5 to 1×10^6 cells in the case of DAPI and for 1×10^6 cells in the case of PI/RNase. When testing the growth density of the cells, the number of cells seemed to vary by a large margin and it was assumed that the collection method was inaccurate and could give wide variations in cell count, and that even if there were enough cells, most of them would be lost during sample preparation. After several samples (Appendixes B1, B2, and B3) had been analysed by the flow cytometer, it was found that the wells did yield enough for analysis as each sample only needed a minimum of 10,000 cells, and that during sample preparation the number of cells lost did not fall below that minimum. This number was based on sampling the tube on the unlimited setting. The cell suspension in the tube would run out at between 20,000 and 40,000 events depending on the sample.

Increasing the amount of time that the cells were centrifuged for during cell splitting from 3 minutes in the original protocol to 6 minutes in the final protocol increased the number of cells that could be transplanted into the new flask. It is believed that this is due to the longer amount of time the cells had to form a pellet at the bottom of the Falcon tube. Changing the size of the tube from 50 mL to 15 mL also resulted in the formation of a more distinct pellet, allowing for the removal of more of the supernatant from the tube with a lower risk of disturbing the pellet.

Changing the amount of time that the cells were trypsinated for was changed from up to 10 minutes to only up to 2 minutes. This was preferable because it decreased the amount of time that the cells were being stressed by the Trypsin, something that could potentially harm the cells. It could also potentially affect results from the analysis of temperature stress on the cells. It also decreased the overall amount of time that the protocol took. Also, slapping the side of the cell

flasks/microplates was deemed more effective, as the sudden impact could dislodge the cells more effectively than slightly tapping the cells for minutes at a time could. The slap did not seem to have a negative impact on the cells, as they continued to grow normally, and any sample prepared did not show any sign of increased stress or increase in cellular debris.

4.2 Optimisation of protocol for cell cycle testing

Increasing the amount of time that the cells were centrifuged after fixation with ethanol from 3 minutes to 6 minutes at speed and from 1200 rpm to 2000 rpm, as was done between Appendix A18 and Appendix A41, increased the yield of cells that could be obtained from each well. The pellet that formed in the tube was larger and of a more even shape. There were less free-floating fragments that were unable to join the larger pellet at the bottom of the tube. This also meant that it was less likely to disturb the pellet when removing the supernatant as the pellet was larger and clearer. The changes in the fixation were tested to see if it gave clearer and more consistent results. When it was increased from 15 minutes to 2 hours, the effects were minimal, but when fixating for more than 2 hours, the cell cycle could be seen more clearly. This could have been due to the prolonged time the cell membrane had to perforate, thereby increasing permeability. The S phase was more visible after 24 hours of fixation when compared with samples fixated for up to 2 hours, as can be seen in Appendixes B33 and B30. In this way, the ethanol had a greater effect. Letting the cells fix in ethanol for greater amounts of time without degrading also meant that the samples could be stored like this over prolonged periods of time, over a week.

After incubating the samples in the dark for more than 1 hour, the peaks that formed corresponding to the cell cycle were clearer and more pronounced than with samples that were only incubated for 30 minutes, as seen in plot 3 in Appendixes B44 and B46. This is believed to be due to the prolonged amount of time that the nucleic acid stains had to bind to the DNA. It was seen that with longer exposure to the stains, the different stages of the cell cycle could be better distinguished. Staining for 2 hours was chosen for the optimised method because it gave clearer results without harming the samples.

The volume of stains used per sample was changed from those specified by the protocols provided by the Thermo Fisher Scientific kits (Appendix 11 and Appendix 12), this was done due to the samples containing fewer than 1 x 10^6 cells, the upper limit that the volumes that the kits specified. In the case of DAPI this volume was reduced from 1.0 mL to 0.5 mL as a standard early in the experiments, as can be seen in the protocol explained in Appendix A22 and on. Reducing the volume of stains that were added to each sample was attempted to reduce the overall waste of stain if large amounts were not needed per sample. The results seemed to have no discernible effect caused by this reduction in stain volume, even when reduced to 0.25 mL of DAPI and 0.25 of PI/RNase, totalling a volume of 0.5 mL of stain in each sample. Prolonging the amount of time that these lover volumes had to stain was also attempted as to mitigate any potential affects in the case the lower volumes did cause an effect.

There were no discernible differences between fixating samples with 96% ethanol and 70% ethanol. It was concluded that using 96% ethanol was more practical and that there would be a lower risk of the formation of ice crystals that could damage the samples.

Samples prepared according to the methods described in Appendixes A23, A24, and A25 were collected with use of a cell scraper in addition to Trypsin. The use of cell scrapers was attempted in an effort to reduce the effects of Trypsin, under the assumption that the Trypsin was stressing the cells and leading to the lack of a distinct cell cycle in the results from the flow cytometer. Also, the old medium and HBSS was added to the samples instead of being discarded. This meant that all the free floating and dead cells were added to the final sample, thus increasing the amount of cellular debris that could be found in the flow cytometer. These methods were decided against, as they did not add any information to the cell cycle, but rather added more noise, as can be seen in the results in Appendixes B20 and B21. These protocols also gave results in which the total amount of events recorded by the flow cytometer were lower than 10,000, the recommended minimum for analysis. This could have been due to the samples being diluted when the old medium and the HBSS were added to the collection tube. The higher volume of cell suspension did not have enough time to form a cell pellet due to the higher volume of liquid the cells would have to travel through during the centrifugation process (step 9 in Appendixes A23, A24, and A25). If the centrifugation time had been increased to over 6 minutes, this problem

could have been avoided but ultimately it was decided not to increase the time because as it could have increased cellular debris. If more events had been analysed in the flow cytometer, numbers above the minimum of 10,000, it could have potentially reduced the amount of noise. Nevertheless, the ratio of dead cells and cellular debris to live cells would be higher in samples in which the old medium was collected and analysed.

4.3 Deciding on an optimised method

The use of DAPI and PI/RNase for staining the same sample did not appear to effect the clarity of the results when viewed in the flow cytometer, but it was ultimately decided to use PI/RNase alone for the optimised method, as using this stain had yielded a consistently clear view of the cell cycle in most of the protocols in which it was used. Staining the sample with DAPI resulted in a clear area of cellular debris/fragmented DNA, but there was no clear indication of an area corresponding to the cell cycle. There was only a large peak of unknown events, as can be seen in plots 9 and 10 in Appendix B36. The cell cycle is clearly visible in plots 2 and 3 in Appendix B36, which show the light registered from PI.

A further reason for the decision to use only PI/RNase was that it came ready for use, and therefore all that had to be done was to add it directly to the sample and incubate. By contrast, DAPI had to be prepared from a stock solution and a buffer. The buffer had to be made separately in the lab. This meant that there was a higher likelihood of faulty preparation of both the buffer itself and of the working solution. Two buffer solutions were made for testing with DAPI, as explained in Appendixes A13 and A14. The difference between them was that one contained TWEEN® 20 and the other contained Nonidet P40. The protocol that came with the Thermo Fisher Scientific DAPI kit (Appendix A11) specified for the use of Nonidet P40, but due to the lack of it in the lab, it was substituted with TWEEN® 20. Thermo Fisher Scientific could not guarantee the effectiveness of TWEEN® 20. The results from the flow cytometer did not show any apparent difference between these two detergents, as can be seen in Appendixes B5 and B8. Nevertheless, it was opted to use the Nonidet P40 version (Appendix A16) in order to eliminate the potential effect that the TWEEN® 20 version (Appendix A15) could have had on the samples. Also, the use of DAPI was decided against because it would have added additional steps to the procedure and thereby increased the potential for human error in the preparation of

the samples. Using two different stains on the same sample was deemed unnecessary and counterproductive for the optimised protocol.

After looking back at all of the results obtained by using all the different protocols, as seen in the whole of Appendix B, it was decided to use a longer centrifugation time at a higher rpm in the case of the centrifugation after fixation, the use of 96% ethanol, and longer fixation and staining times gave better and more consistent results. The overall result is in the protocol explained in Appendix A41.

4.4 Cell cycle stress analysis

As a main observation, most of the samples incubated at 26 °C showed a higher amount of cellular debris in the flow cytometer analysis compared with the cell samples incubated at 20 °C, as seen in Appendix D50. This observation clearly indicates that a higher incubation temperature did stress the cells as their normal optimal growing conditions were disturbed. A reason for this could be that cells' natural mechanisms for dealing with stress are not as efficient over prolonged periods of time, when proteins are denatured, and the cellular integrity is affected. There is one data point on the graph (Appendix A50) that deviates from this trend of higher cellular debris at 26 °C, and this is at the samples collected at 42 hours, where the levels of cellular debris were higher in those samples grown at 20 °C. This could be an isolated scenario as this is the only point on the graph where this happens, or it could be a part of trend that could not be seen due both to the large amount of time between each sample and to there being so few samples taken at each time point.

The G1 phase graph (Appendix D41) shows a great difference in the number of cells between 8 and 26 hours. It is thought that this large difference between the control cell and the stressed cells at 26 hours might have been caused by random variation due to the large variation between the two points for 26 hours. It is not possible to conclude anything for certain, as there were only three parallels for each point and because there was a large amount of time between the 8-hour and 26-hour samples. The values for 0 hours to 8 hours are very similar in the graph. There is

one outlier and that is at 6 hours for 26 °C. This is thought to be an incorrect result as it deviates so much from both the values for 20 °C and 26 °C around it. After 42 hours of exposure the two graphs in the charts start to line up again. This could be due to the cells incubated at 26 °C adapting to the higher temperatures.

In the same time period, between 8 hours and 26 hours, the number of stressed cells at 26 °C found in the S phase was higher than the number of the control cells at 20 °C. This could indicate a cell number increase due to the possibility that cells in the G1 phase were no longer in arrest, and therefore they continued with their growth and proliferation through the cell cycle. After 42 hours of exposure to 26 °C, the cell numbers began to line up with the control cells, with the cell number increasing after 52 hours. Again, more data should be collected between 8 hours and 26 hours and the number of parallels increased for more reliable results.

Cells in the G2/M phase at 20 °C and 26 °C seem to follow the same trends from 0 hours to 30 hours, being arrested at the same time points (8 hours and 30 hours). After 30 hours, the cell arrest at 26 °C is prolonged compared with the cells at 20 °C, as at 42 hours the cell number at 26 °C is higher than the control cell number at 20 °C. This can indicate that the increase in temperature might have influenced the cellular arrest in the G2/M phase.

In many of the results that can be found in Appendix list B and C there is a visible area on the graph showing the cell cycle that does not relate to any of the phases (G1, S, and G2/M). This area is found to the right of the G2/M phase as can be seen in plot 8 of figure 16 and in plot 2 of figure 19. This area of the graph is thought to represent clusters of cells, thus making them contain larger amounts of DNA and being larger than cells in the G2/M phase.

Due to problems with the BD Accuri™ Cytometer, the samples incubated at 20 °C and 26 °C for 18 hours (T5) and 22 hours (T6) were lost. Therefore, these samples did not contribute any data to the results.

No further conclusions based on the data from the stress analysis could be made, due both to the large intervals of time between sampling and to the samples consisting of only three parallels. It is advised that to obtain more conclusive results, the number of parallels should be increased and the amount of time between sample collection reduced.

5 Conclusions

After attempting multiple different protocols, a method for the analysis of the cell cycle in CHSE-214 cell in the BD Accuri™ Cytometer was optimised.

Due to low numbers of parallels and large gaps in the time between sample collection, and problems with the BD Accuri™ Cytometer, no meaningful conclusions could be drawn about the effects of temperature stress on CHSE-214 cells. Although from the small amount of data that each point gives there does seem to be a change in the cell cycle of both control (20 °C) and stressed cells (26 °C) between 8 and 26 hours of exposure. There also appears to be an adaptation that occurs after 42 hours of exposure to 26 °C as the graphs for stressed cells (26 °C) and control cells (20 °C) appear to line up with each other.

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Appendixes

Appendix A

Methods used for the optimisation of a protocol for the study of the cell cycle in CHSE-214 cells using flow cytometry (A1–A41).

Appendix B

Flow cytometry results for the optimisation of a protocol for the study of the cell cycle in CHSE-214 cells using flow cytometry (B1–B49).

Appendix C

Flow cytometry results for the effects of temperature stress on the cell cycle in CHSE-214 cells using flow cytometry (C1–C57).

Appendix D

Results from Appendix C are displaced in tables and graphs (D1-D59).

Appendix A1

Procedure – Preparation of growth medium

Method

1. In LAF-cabinet warm up L-15 medium (Gibco by Life Technologies ™, #31415-029), Fetal Bovine Serum (FBS, Gibco by Life Technologies ™, #A3840401), and Gentamicin to room temperature.
2. Add 50 mL of FBS to the L-15 medium flask.
3. Add 0.5 mL of Gentamicin (Gibco by Life Technologies ™, #15750-037) to L-15 medium flask.
4. Slowly mix the content of the L-15 medium flask by turning the flask on its head several times.
5. Place cell medium in refrigerator for storage.

Appendix A2

Procedure – Splitting and growing CHSE-214 cells in 75cm^2 cell culture flasks, first method

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the flask.
3. Gently rinse the interior of the flask with 2 x 5.0 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 1.0 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait **5 to 10 minutes** at room temperature.
5. **Gently tap the bottle** before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 4.0 mL of L-15 medium to the flask.
7. Gently mix the content of the flask before transferring the content to a **50 mL** Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for **3 minutes**.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Add 1 mL of L-15 medium to the Falcon tube. Gently pipette the medium until the pellet has dissolved.
11. Add 5 mL of L-15 medium to the Falcon tube.
12. Add 17 mL of L-15 medium to 2 new cell flasks (1:2 splitting).
13. Add 3 mL of cell suspension from Falcon tube to each new flask. Gently mix the content. Place the new cell flasks in the incubator at 20 °C.

Appendix A3

Procedure – Splitting and growing CHSE-214 cells in 75cm^2 cell culture flasks, second method

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the flask.
3. Gently rinse the interior of the flask with 2 x 5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 1 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait **5 to 10 minutes** at room temperature.
5. **Gently tap the bottle** before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 4.0 mL of L-15 medium to the flask.
7. Gently mix the content of the flask before transferring the content to a **50 mL** Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for **5 minutes**.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Add 1 mL of L-15 medium to the Falcon tube. Gently pipette the medium until the pellet has dissolved.
11. Add 5 mL of L-15 medium to the Falcon tube.
12. Add 17 mL of L-15 medium to 2 new cell flasks (1:2 splitting).
13. Add 3 mL of cell suspension from Falcon tube to each new flask. Gently mix the content. Place the new cell flasks in the incubator at 20 °C.

Appendix A4

Procedure – Splitting and growing CHSE-214 cells in 75cm^2 cell culture flasks, final method

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the flask.
3. Gently rinse the interior of the flask with 2 x 5.0 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 1.0 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait **1 to 2 minutes** at room temperature.
5. **Gently slap the side of the plate 3 to 5 times** to loosen the cells before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 4.0 mL of L-15 medium to the flask.
7. Gently mix the content of the flask before transferring the content to a **15 mL** Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for **6 minutes**.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Add 1.0 mL of L-15 medium to the Falcon tube. Gently pipette the medium until the pellet has dissolved.
11. Add 5.0 mL of L-15 medium to the Falcon tube.
12. Add 17.0 mL of L-15 medium to 2 new cell flasks (1:2 splitting).
13. Add 3.0 mL of cell suspension from Falcon tube to each new flask. Gently mix the content. Place the new cell flasks in the incubator at 20 °C.

Appendix A5

Procedure – Splitting cells from 75cm^2 cell culture flask to 6 well 9.6 cm^2 microplate, first method

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the flask.
3. Gently rinse the interior of the flask with 2 x 5.0 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 1.0 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait **5 to 10 minutes** at room temperature.
5. **Gently tap the bottle** before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 4.0 mL of L-15 medium to the flask.
7. Gently mix the content of the flask before transferring the content to a **50 mL** Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for **3 minutes**.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Add 1 mL of L-15 medium to the Falcon tube. Gently pipette the medium until the pellet has dissolved.
11. Add 5.0 mL of L-15 medium to the Falcon tube.
12. Add 1.0 mL of L-15 medium to each well of the microplate (1:6 splitting).
13. Add 1.0 mL of cell suspension from Falcon tube to each well. Gently mix the content. Place the new cell flasks in the incubator at 20 °C.

Appendix A6

Procedure – Splitting cells from 75cm^2 cell culture flask to 6 well 9.6 cm^2 microplate, second method

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the flask.
3. Gently rinse the interior of the flask with 2 x 5.0 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 1.0 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait **5 to 10 minutes** at room temperature.
5. **Gently tap the bottle** before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 4.0 mL of L-15 medium to the flask.
7. Gently mix the content of the flask before transferring the content to a **50 mL** Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for **5 minutes**.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Add 1.0 mL of L-15 medium to the Falcon tube. Gently pipette the medium until the pellet has dissolved.
11. Add 5.0 mL of L-15 medium to the Falcon tube.
12. Add 1.0 mL of L-15 medium to each well of the microplate (1:6 splitting).
13. Add 1.0 mL of cell suspension from Falcon tube to each well. Gently mix the content. Place the new cell flasks in the incubator at 20 °C.

Appendix A7

Procedure – Splitting cells from 75cm^2 cell culture flask to 6 well 9.6 cm^2 microplate, third method

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the flask.
3. Gently rinse the interior of the flask with 2 x 5.0 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 1.0 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. **Wait 1 to 2 minutes** at room temperature.
5. **Gently slap the side of the plate 3 to 5 times** to loosen the cells before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 4.0 mL of L-15 medium to the flask.
7. Gently mix the content of the flask before transferring the content to a **15 mL** Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for **6 minutes**.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Add 1.0 mL of L-15 medium to the Falcon tube. Gently pipette the medium until the pellet has dissolved.
11. Add 5.0 mL of L-15 medium to the Falcon tube.
12. Add 1.0 mL of L-15 medium to each well of the microplate (1:6 splitting).
13. Add 1.0 mL of cell suspension from Falcon tube to each well. Gently mix the content. Place the new cell flasks in the incubator at 20 °C.

Appendix A8

Procedure – Assaying cells growth density in 75cm^2 cell culture flasks

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the flask.
3. Gently rinse the interior of the flask with 2 x 5.0 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 1.0 mL Trypsin (Gibco by Life Technologies ™, #25200-072) to the well. Wait 5 to 10 minutes at room temperature.
5. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 5.0 mL of L-15 medium to flask (making the total amount 6.0 mL). Gently mix the content.
7. Transfer 10.0 µL of cell suspension to both chambers of the Bürker counting chamber using a pipette and filtered pipette tips.
8. Place counting chamber under microscope and count the number of cells using the formula; C=N/(AVF)*1000.

Appendix A9

Procedure – Assaying cells growth density in 6 well 9.6 cm^2 microplate

Method

1. Check confluence of cells using an inverted microscope.
2. Add 0.2 mL Trypsin (Gibco by Life Technologies ^{TM}, #25200-072) to the well (Making the total volume of the well 2 mL). Wait 5 to 10 minutes at room temperature.
3. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
4. Add 0.8 mL of medium to well. Gently mix the content.
5. Transfer 10 µL of cell suspension to both chambers of the Bürker counting chamber using a pipette and filtered pipette tips.
6. Place counting chamber under microscope and count the number of cells using the formula; C=N/(AVF)*1000.

Appendix A10

Procedure –DAPI Stock solution

Method

1. Make a 5 mg/mL DAPI (Invitrogen ™, # D3571) stock solution (14.3 mM) by dissolving the contents of one vial (10 mg) in 2 mL of deionized water (dH_2O). This is done once, and the vial stored dark in a refrigerator.

Appendix A11

Procedure – Thermo Fisher DAPI protocol

Method

1. Collect a cell suspension of 2×10^5 to 1×10^6 cells.
2. Pellet the cells by centrifugation and discard the supernatant.
3. Tap the tube to resuspend the pellet in the residual liquid and add 1 mL of PBS at room temperature.
4. Transfer the full volume of resuspended cells to 4mL of 96% ethanol at –20 °C by pipetting the cell suspension slowly into the ethanol while vortexing at top speed. Leave the cells in ethanol at –20 °C for 5–15 minutes.
5. Pellet the cells by centrifugation and discard the ethanol
6. Tap the tube to loosen the pellet and add 5 mL of PBS at room temperature. Allow the cells to rehydrate for 15 minutes.
7. Dilute the DAPI (Invitrogen ™, # D3571) stock solution to 3 μM in staining buffer (100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM CaCl2, 0.5 mM MgCl2, 0.1% Nonidet P-40). A 1 mL volume will be required for each cell sample.
8. Centrifuge the cell suspension (from step 7) and discard the supernatant. Tap to loosen the pellet and add 1 mL of DAPI diluted in staining buffer.
9. Incubate for 15 minutes at room temperature.
10. Analyse by flow cytometry in the presence of the dye. If the cells are to be viewed by fluorescence microscopy, centrifuge the sample, remove the supernatant and resuspend cells in fresh buffer. Apply a drop of the suspension to a microscope slide, cover with a coverslip and view.

Appendix A12

Procedure – Thermo Fisher PI/RNase protocol

Method

1. Harvest the cell sample(s).
2. Fix cells according to your preferred protocol.
3. Wash the cells. All fixative should be removed from cells before proceeding with cell staining.
4. Prepare flow cytometry samples each containing ~ 1×10^6 cells in suspension
5. Centrifuge the samples and decant the supernatant, leaving a pellet of cells in each sample tube.
6. Add 0.5 mL of FxCycleTM PI/RNase Staining Solution stain to each flow cytometry sample, mix well.
7. Incubate the samples for 15–30 minutes at room temperature, protected from light
8. Analyse the samples without washing, using 488-nm, 532-nm, or similar excitation, and collect emission using a 585/42 bandpass filter or equivalent.

Appendix A13

Procedure – Preparing 1 litre of staining buffer Tween 20 version

Method

1. To an Erlenmeyer flask add 600 mL of MilliQ water.
2. Place the flask on a magnetic heating plate. Place a stirring magnet in the flask to stir the content.
3. Add 12.112 g of Tris, 8.77 g of NaCl, 0.111 g of $CaCl_2$, 0.102 g of $MgCl_2$, and **1.0 mL of Tween 20** to the flask. Stir until the solution is clear.
4. Check the pH of the solution. Using 1 M HCl and 1 M NaOH change the pH of the solution to 7.4.
5. Transfer content of the Erlenmeyer flask to a 1.0 L volumetric flask.
6. Add MilliQ water to the solution so that it equals 1.0 L.
7. Transfer the solution to a 1.0 L bottle with screw cork. Place the bottle in storage at 4 °C.

Appendix A14

Procedure – Preparing 1 litre of staining buffer Nonidet P-40 version

Method

1. To an Erlenmeyer flask add 600 mL of MilliQ water.
2. Place the flask on a magnetic heating plate. Place a stirring magnet in the flask to stir the content.
3. Add 12.112 g of Tris, 8.77 g of NaCl, 0.111 g of $CaCl_2$, 0.102 g of $MgCl_2$, and **1.0 mL of Nonidet P-40** to the flask. Stir until the solution is clear.
4. Check the pH of the solution. Using 1 M HCl and 1 M NaOH change the pH of the solution to 7.4.
5. Transfer content of the Erlenmeyer flask to a 1.0 L volumetric flask.
6. Add MilliQ water to the solution so that it equals 1.0 L.
7. Transfer the solution to a 1.0 L bottle with screw cork. Place the bottle in storage at 4 °C.

Appendix A15

Procedure – Preparing DAPI working solution Tween 20 version

Method

1. Prepare DAPI (Invitrogen ™, # D3571) working solution by diluting the DAPI stock solution to 3 μM in staining buffer to make the required amount of working solution (100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM CaCl2, 0.5 mM MgCl2, **0.1% Tween 20**). Staining buffer referred to in Appendix A13.

Appendix A16

Procedure – Preparing DAPI working solution Nonidet P-40 version

Method

1. Prepare DAPI (Invitrogen ^{TM}, # D3571) working solution by diluting the DAPI stock solution to 3 μM in staining buffer to make the required amount of working solution (100 mM Tris, pH 7.4, 150 mM NaCl, 1 mM CaCl2, 0.5 mM MgCl2, **0.1% Nonidet P-40**). Staining buffer referred to in Appendix A14.

Appendix A17

Procedure – Flow Cytometry calibration

Preparation of material

1. Open the BD Accuri C6 programme
2. Open the "washing and calibration 061118 template" in computer
3. Label 5 small tubes corresponding to the boxes A1-A5 in the calibration programme (A1, cleaning; A2, decontamination; A3, MilliQ; A4, 8 peak; A5, 6 peak)
4. Add 3 mL of 'cleaning' solution into A1
5. Add 3 mL of 'decontamination' solution into A2
6. Add 3 mL of MilliQ into A3
7. Add ½ mL of MilliQ into A4 and A5
8. Add 1 drop of 8 peak beads into A4
9. Add 1 drop of 6 peak beads into A5

Calibration of A1-A5 tubes

1. Place A1 tube in the Flow Cytometer, set the "Fluidics" at fast speed, set "Run with limits" at 5 minutes, then "Run"
2. Follow the same procedure as A1 for A2 and A3 too
3. Place A4 tube in the Flow Cytometer, set the "Fluidics" at slow speed, set "Run with limits" at 10 000 events, then "Run" - the events/second must be at around 200
4. Remove the A4 tube and "Backflush" twice with a beaker underneath to collect the excess
5. Follow the same procedure as A4 for A5
6. Remove the A5 tube and "Backflush" twice with a beaker underneath to collect the excess
7. Place A3 tube in the Flow Cytometer, set the "Fluidics" at fast speed, set "Run with limits" at 5 minutes, then "Run"

The Flow Cytometer is now calibrated

Appendix A18

Procedure – DAPI staining

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the well.
3. Gently rinse the interior of the flask with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait 5 to 10 minutes at room temperature.
5. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well.
7. Gently mix the content of the wells before transferring the content to a 15 mL Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Take falcon tube out of LAF-cabinet
11. Resuspend pellet with 1 mL PBS at room temperature.
12. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 4.0 mL of 96% ethanol at –20 °C while vortexing at full speed.
13. Let the cells fix for 10 to 15 minutes in a fridge at –20 °C.
14. Centrifuge the Falcon tube at 1200 rpm for **3 minutes**.
15. Remove and discard the ethanol.
16. Tap the tube to loosen the pellet.
17. Add 5.0 mL of PBS at room temperature. Allow to rehydrate for 15 minutes.
18. Prepare staining the required amount of DAPI (Invitrogen ™, # D3571) working solution according to appendix 15.
19. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
20. Tap the tube to loosen the pellet.
21. Add 1.0 mL of DAPI working solution to cell suspension.
22. Incubate for 15 minutes at room temperature in the dark.
23. Analyse the sample by flow cytometry.

Appendix A19

Procedure – DAPI staining

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the well.
3. Gently rinse the interior of the flask with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait 5 to 10 minutes at room temperature.
5. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well.
7. Gently mix the content of the flask before transferring the content to a 15 mL Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Take falcon tube out of LAF-cabinet.
11. Resuspend pellet with 1 mL PBS at room temperature.
12. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 4 mL of 96% ethanol at –20 °C while vortexing at full speed.
13. Let the cells fix for 10 to 15 minutes in a fridge at –20 °C.
14. Centrifuge the Falcon tube at 1200 rpm for **5 minutes**.
15. Remove and discard the ethanol.
16. Tap the tube to loosen the pellet.
17. Add 5 mL of PBS at room temperature. Allow to rehydrate for 15 minutes.
18. Prepare the required amount of DAPI (Invitrogen ™, # D3571) working solution according to appendix A15.
19. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
20. Tap the tube to loosen the pellet.
21. Add 1.0 mL of DAPI working solution to cell suspension.
22. Incubate for 15 minutes at room temperature in the dark.
23. Analyse the sample by flow cytometry.

Appendix A20

Procedure – PI/RNase staining

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the well.
3. Gently rinse the interior of the flask with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait 5 to 10 minutes at room temperature.
5. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well.
7. Gently mix the content of the flask before transferring the content to a 15 mL Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Take falcon tube out of LAF-cabinet.
11. Resuspend pellet with 1 mL PBS at room temperature.
12. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 4 mL of 96% ethanol at –20 °C while vortexing at full speed.
13. Let the cells fix for 10 to 15 minutes in a fridge at –20 °C.
14. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
15. Remove and discard the ethanol.
16. Tap the tube to loosen the pellet.
17. Add 5 mL of PBS at room temperature. Allow to rehydrate for 15 minutes.
18. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
19. Tap the tube to loosen the pellet.
20. Add 0.5 mL of PI/RNase staining solution (Invitrogen ™, # F10797).
21. Incubate for 15-30 minutes in the dark.
22. Analyse the sample by flow cytometry.

Appendix A21

Procedure – DAPI staining

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the well.
3. Gently rinse the interior of the flask with 2 x 0.50 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait 5 to 10 minutes at room temperature.
5. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well.
7. Gently mix the content of the flask before transferring the content to a 15 mL Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Take falcon tube out of LAF-cabinet.
11. Resuspend pellet with 1.0 mL PBS at room temperature.
12. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 4.0 mL of 96% ethanol at –20 °C while vortexing at full speed.
13. Let the cells fix for 10 to 15 minutes in a fridge at –20 °C.
14. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
15. Remove and discard the ethanol.
16. Tap the tube to loosen the pellet.
17. Add 5.0 mL of 1 x PBS at room temperature. Allow to rehydrate for 15 minutes.
18. Prepare the required amount of DAPI (Invitrogen ™, # D3571) working solution according to appendix A16
19. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
20. Tap the tube to loosen the pellet.
21. Add 1.0 mL of DAPI working solution to cell suspension.
22. Incubate for 15 minutes at room temperature in the dark.
23. Analyse the sample by flow cytometry.

Appendix A22

Procedure – DAPI&PI/RNase

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the well.
3. Gently rinse the interior of the flask with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait 5 to 10 minutes at room temperature.
5. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well.
7. Gently mix the content of the flask before transferring the content to a 15 mL Falcon tube.
8. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
9. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
10. Take falcon tube out of LAF-cabinet.
11. Resuspend pellet with 1 mL 1x PBS at room temperature.
12. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 4 mL of 96% ethanol at –20 °C while vortexing at full speed.
13. Let the cells fix for 10to 15 minutes in a fridge at –20 °C.
14. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
15. Remove and discard the ethanol.
16. Tap the tube to loosen the pellet.
17. Add 5 mL of 1x PBS at room temperature. Allow to rehydrate for 15 minutes.
18. Prepare the required amount of DAPI (Invitrogen ™, # D3571) working solution according to appendix A16
19. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
20. Tap the tube to loosen the pellet.
21. Add 0.5 mL of DAPI working solution to cell suspension.
22. Add 0.5 mL of PI/RNase staining solution (Invitrogen ™, # F10797).
23. Incubate for 30 minutes at room temperature in the dark.
24. Analyse the sample by flow cytometry.

Appendix A23

Procedure – DAPI staining, collection with cell scrape

Method

1. Check confluence of cells using an inverted microscope.
2. Use a cell scrape to loosen cells from well surface.
3. Transfer the old L-15 medium from the well into a 15 mL Falcon tube.
4. Gently rinse the interior of the flask with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), transfer the HBSS to the Falcon tube.
5. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait 5 to 10 minutes at room temperature.
6. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
7. Add 0.8 mL of L-15 medium to the well.
8. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
9. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
10. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
11. Take the Falcon tube out of LAF-cabinet.
12. Resuspend pellet with 1 mL 1x PBS at room temperature.
13. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 4 mL of 96% ethanol at –20 °C while vortexing at full speed.
14. Let the cells fix for 10-15 minutes in a freezer at –20 °C.
15. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
16. Remove and discard the ethanol.
17. Tap the tube to loosen the pellet.
18. Add 5 mL of 1x PBS at room temperature. Allow to rehydrate for 15 minutes. Vortex for a few seconds.
19. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
20. Tap the tube to loosen the pellet.
21. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
22. Add 1.0 mL of DAPI working solution to cell suspension.
23. Incubate for 15 minutes at room temperature in the dark.
24. Analyse the sample by flow cytometry.

Appendix A24

Procedure – PI/RNase staining, collection with cell scrape

Method

1. Check confluence of cells using an inverted microscope.
2. Use a cell scrape to loosen cells from well surface.
3. Transfer the old L-15 medium from the well into a 15 mL Falcon tube.
4. Gently rinse the interior of the flask with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), transfer the HBSS to the Falcon tube.
5. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait 5 to 10 minutes at room temperature.
6. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
7. Add 0.8 mL of L-15 medium to the well.
8. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
9. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
10. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
11. Take the Falcon tube out of LAF-cabinet.
12. Resuspend pellet with 1 mL 1x PBS at room temperature.
13. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 4 mL of 96% ethanol at –20 °C while vortexing at full speed.
14. Let the cells fix for 10-15 minutes in a freezer at –20 °C.
15. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
16. Remove and discard the ethanol.
17. Tap the tube to loosen the pellet.
18. Add 5 mL of 1x PBS at room temperature. Allow to rehydrate for 15 minutes. Vortex for a few seconds.
19. Centrifuge the cell suspension. Remove the supernatant. Tap the tube to loosen the pellet.
20. Add 0.5 mL of PI/RNase staining solution (Invitrogen ™, # F10797).
21. Incubate for 15-30 minutes at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A25

Procedure – DAPI&PI/RNase staining, collection with cell scrape

Method

1. Check confluence of cells using an inverted microscope.
2. Use a cell scrape to loosen cells from well surface.
3. Transfer the old L-15 medium from the well into a 15 mL Falcon tube.
4. Gently rinse the interior of the flask with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), transfer the HBSS to the Falcon tube.
5. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the flask. Distribute the Trypsin over all the cells. Wait 5 to 10 minutes at room temperature.
6. Gently tap the bottle before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
7. Add 0.8 mL of L-15 medium to the well.
8. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
9. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
10. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
11. Take the Falcon tube out of LAF-cabinet.
12. Resuspend pellet with 1 mL 1x PBS at room temperature.
13. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 4 mL of 96% ethanol at –20 °C while vortexing at full speed.
14. Let the cells fix for 10-15 minutes in a freezer at –20 °C.
15. Centrifuge the Falcon tube at 1200 rpm for 5 minutes.
16. Remove and discard the ethanol.
17. Tap the tube to loosen the pellet.
18. Add 5 mL of 1x PBS at room temperature. Allow to rehydrate for 15 minutes. Vortex for a few seconds.
19. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
20. Tap the tube to loosen the pellet.
21. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
22. Add 0.5 mL of DAPI working solution to cell suspension.
23. Add 0.5 mL of PI/RNase staining solution (Invitrogen ™, # F10797.
24. Incubate for 30 minutes at room temperature in the dark.
25. Analyse the sample by flow cytometry.

Appendix A26

Procedure – A-series 15-minute fixation, 0.50 mL staining, & 30-minute incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2 mL of 96% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for **15 minutes** in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.50 mL** of DAPI working solution to cell suspension.
20. Add **0.50 mL** of PI/RNase staining solution (Invitrogen ™, # F10797 to cell suspension.
21. Incubate for **30 minutes** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A27

Procedure – A-series 15-minute fixation, 0.25 mL staining, & 2-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2 mL of 96% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for **15 minutes** in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **2 hours** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A28

Procedure – A-series 1-hour fixation, 0.50 mL staining, & 30-minute incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2 mL of 96% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for **1 hour** in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.50 mL** of DAPI working solution to cell suspension.
20. Add **0.50 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **30 minutes** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A29

Procedure – A-series 1-hour fixation with 96% ETOH, 0.25 mL staining, & 2-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of **96% ethanol** at –20 °C while vortexing at full speed.
12. Let the cells fix for **1 hour** in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **2 hours** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A30

Procedure – A-series 2-hour fixation with 96% ETOH, & 0.50 mL staining, & 30-minute incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of **96% ethanol** at –20 °C while vortexing at full speed.
12. Let the cells fix for **2 hours** in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.50 mL** of DAPI working solution to cell suspension.
20. Add **0.50 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **30 minutes** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A31

Procedure – A-series 2-hour fixation with 96% ETOH, 0.25 mL staining, & 2-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of **96% ethanol** at –20 °C while vortexing at full speed.
12. Let the cells fix for **2 hours** in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **2 hours** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A32

Procedure – B-series 24-hour fixation with 96% ETOH, 0.50 mL staining, & 30-minute incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of **96% ethanol** at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.50 mL** of DAPI working solution to cell suspension.
20. Add **0.50 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **30 minutes** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A33

Procedure – B-series 24-hour fixation with 96% ETOH, 0.25 mL staining, & 1-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of **96% ethanol** at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **1 hour** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A34

Procedure – B-series 24-hour fixation with 96% ETOH, 0.25 mL staining, & 2-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of **96% ethanol** at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 1200 rpm for 5 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **2 hours** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A35

Procedure – C-series, 24-hour fixation with 70% ETOH, 0.50 mL staining, & 30-minute incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of 70% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at **1200 rpm** for **5 minutes**.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.50 mL** of DAPI working solution to cell suspension.
20. Add **0.50 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **30 minutes** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A36

Procedure – C-series, 24-hour fixation with 70% ETOH, 0.25 mL staining, & 1-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of 70% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at **1200 rpm** for **5 minutes**.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **1 hour** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A37

Procedure – C-series, 24-hour fixation with 70% ETOH, 0.25 mL staining, & 2-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of 70% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at **1200 rpm** for **5 minutes**.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **2 hours** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A38

Procedure – D-series, 24-hour fixation with 70% ETOH, 0.50 mL staining, & 30-minute incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of 70% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at **2000 rpm** for **6 minutes**.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.50 mL** of DAPI working solution to cell suspension.
20. Add **0.50 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **30 minutes** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A39

Procedure – D-series, 24-hour fixation with 70% ETOH, 0.25 mL staining, & 1-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of 70% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at **2000 rpm** for **6 minutes**.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **1 hour** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A40

Procedure – D-series, 24-hour fixation with 70% ETOH, 0.25 mL staining, & 2-hour incubation

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently hit the microplate before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 2.0 mL of 70% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at **2000 rpm** for **6 minutes**.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Prepare DAPI (Invitrogen ™, # D3571) working solution as explained in Appendix A16.
19. Add **0.25 mL** of DAPI working solution to cell suspension.
20. Add **0.25 mL** of PI/RNase staining solution (Invitrogen ™, # F10797) to cell suspension.
21. Incubate for **2 hours** at room temperature in the dark.
22. Analyse the sample by flow cytometry.

Appendix A41

Procedure – Optimized method for sample preparation and analysis with PI

Method

1. Check confluence of cells using an inverted microscope.
2. Remove and discard old L-15 medium from the microplate.
3. Gently rinse the interior of the microplate with 2 x 0.5 mL of HBSS (HyClone ™, #SH30588.02), remove and discard residues of HBSS.
4. Add 0.2 mL of Trypsin (Gibco by Life Technologies ™, #25200-072) to the microplate. Distribute the Trypsin over all the cells. Wait 1 to 2 minutes at room temperature.
5. Gently slap the side of the plate 3 to 5 times to loosen the cells before studying it using an inverted microscope. When at least 80% of the cells have loosened and are free floating, the trypsinization is complete.
6. Add 0.8 mL of L-15 medium to the well. Gently mix the content of the flask before transferring the content to the 15 mL Falcon tube.
7. Centrifuge the Falcon tube at 1200 rpm for 6 minutes.
8. Check that there has formed a pellet in the Falcon tube, then remove the supernatant.
9. Take the Falcon tube out of LAF-cabinet.
10. Resuspend pellet with 1.0 mL 1x PBS at room temperature.
11. Transfer the full volume of resuspended cells by pipetting slowly to a Falcon tube filled with 3.0 mL of 96% ethanol at –20 °C while vortexing at full speed.
12. Let the cells fix for at least 24 hours in a freezer at –20 °C.
13. After fixation centrifuge the Falcon tube at 2000 rpm for 6 minutes.
14. Remove and discard the ethanol. Tap the tube to loosen the pellet. Give time for ethanol to evaporate.
15. Add 3.0 mL of cold 1x PBS at room temperature. Allow to rehydrate for 20 minutes.
16. Centrifuge the Falcon tube at 1200 rpm for 5 minutes. Remove the supernatant.
17. Tap the tube to loosen the pellet.
18. Stain cells by adding 0.50 mL of PI/RNase staining solution (Invitrogen ™, # F10797).
19. Pipette the sample through a strain from the Falcon tube to a sample tube.
20. Incubate for 2 hours at room temperature in the dark.
21. Set Flow cytometer to 40 000 events and set speed to slow.

Appendix B0

All samples were grown for 24 hours at 20 °C. The methods used for each sample is stated in the images caption.

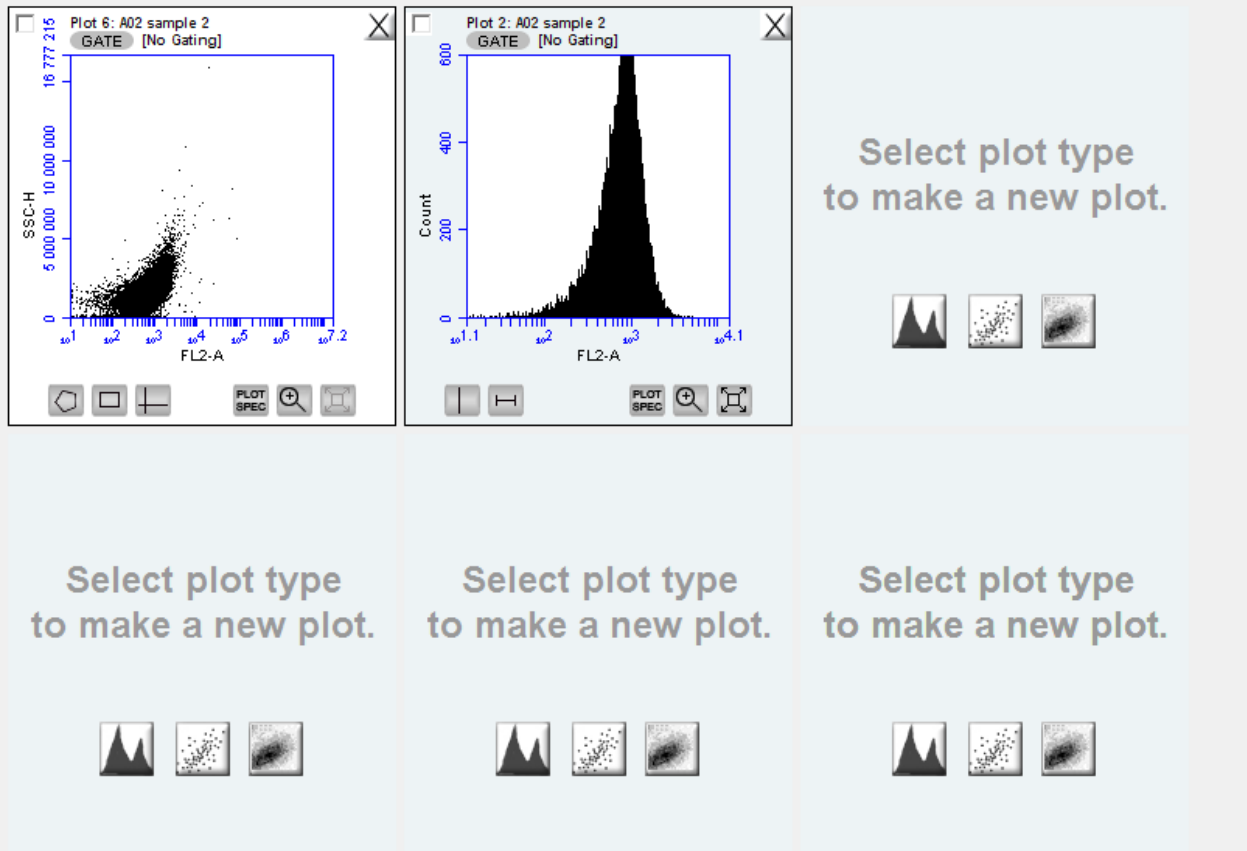
Appendix B1

Plot 6: A01 sample 1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	Mean SSC-H	CV FL2-A	CV SSC-H	Median FL2-A
All	20 000	42	100,00%	100,00%	845,65	1 281 965,71	578,64%	92,45%	

Plot 2: A01 sample 1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	42	100,00%	100,00%	845,65	578,64%	

Figure 28. Sample Nr. 1 Treated with DAPI. Prepared according to Appendix A18.

Appendix B2



Plot 6: A02 sample 2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	Mean SSC-H	CV FL2-A	CV SSC-H	Median FL2-A
All	20 000	40	100,00%	100,00%	845,33	1 624 928,67	128,20%	51,21%	

Plot 2: A02 sample 2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	40	100,00%	100,00%	845,33	128,20%	

Figure 29. Sample Nr. 2. Treated with DAPI. Prepared according to Appendix A18.

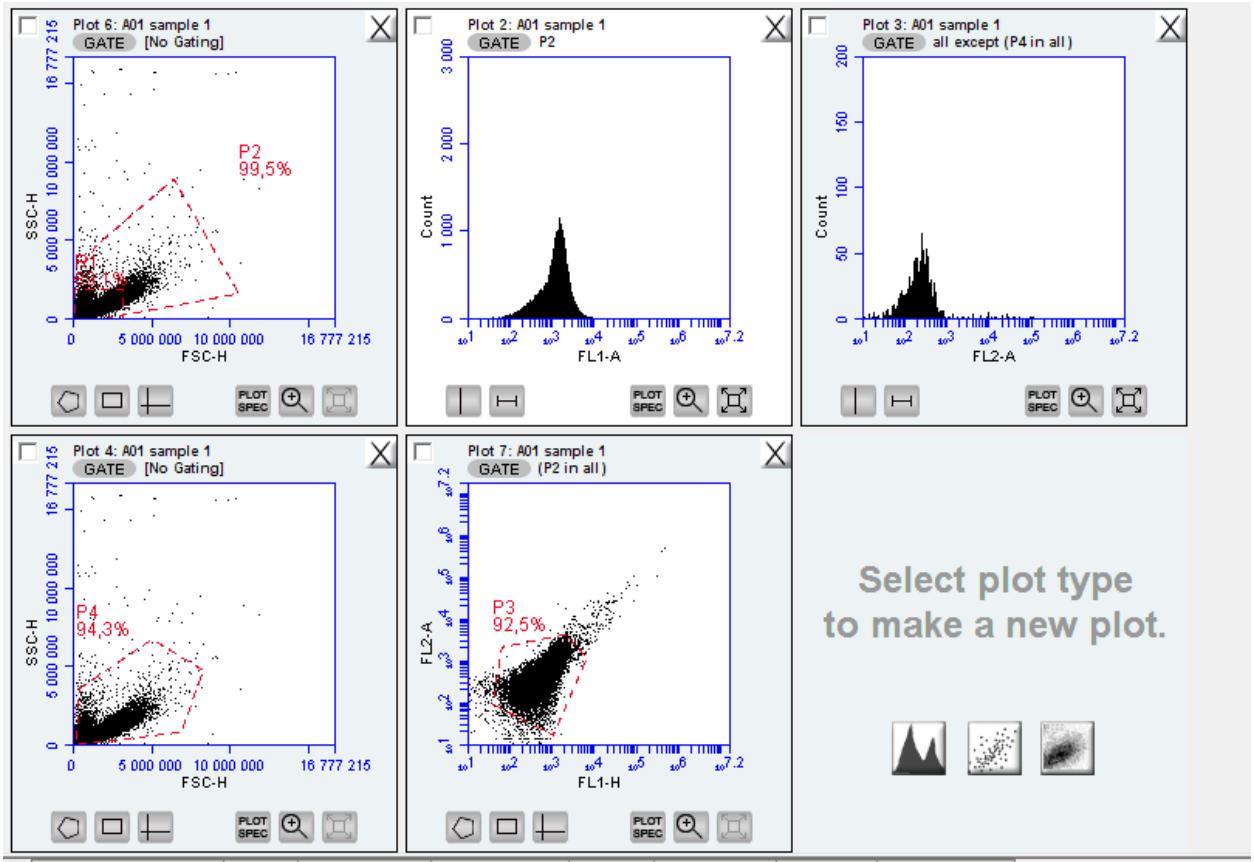
Appendix B3

Plot 6: B02	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	Mean SSC-H	CV FL2-A	CV SSC-H	Median FL2-A	Median SSC-H
All	20 000	58	100,00%	100,00%	829,76	1 558 452,98	93,82%	55,78%		

Plot 2: B02	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	58	100,00%	100,00%	829,76	93,82%	

Figure 30. Sample Nr. 2 repeated. Treated with DAPI. Prepared according to Appendix A18.

Appendix B4



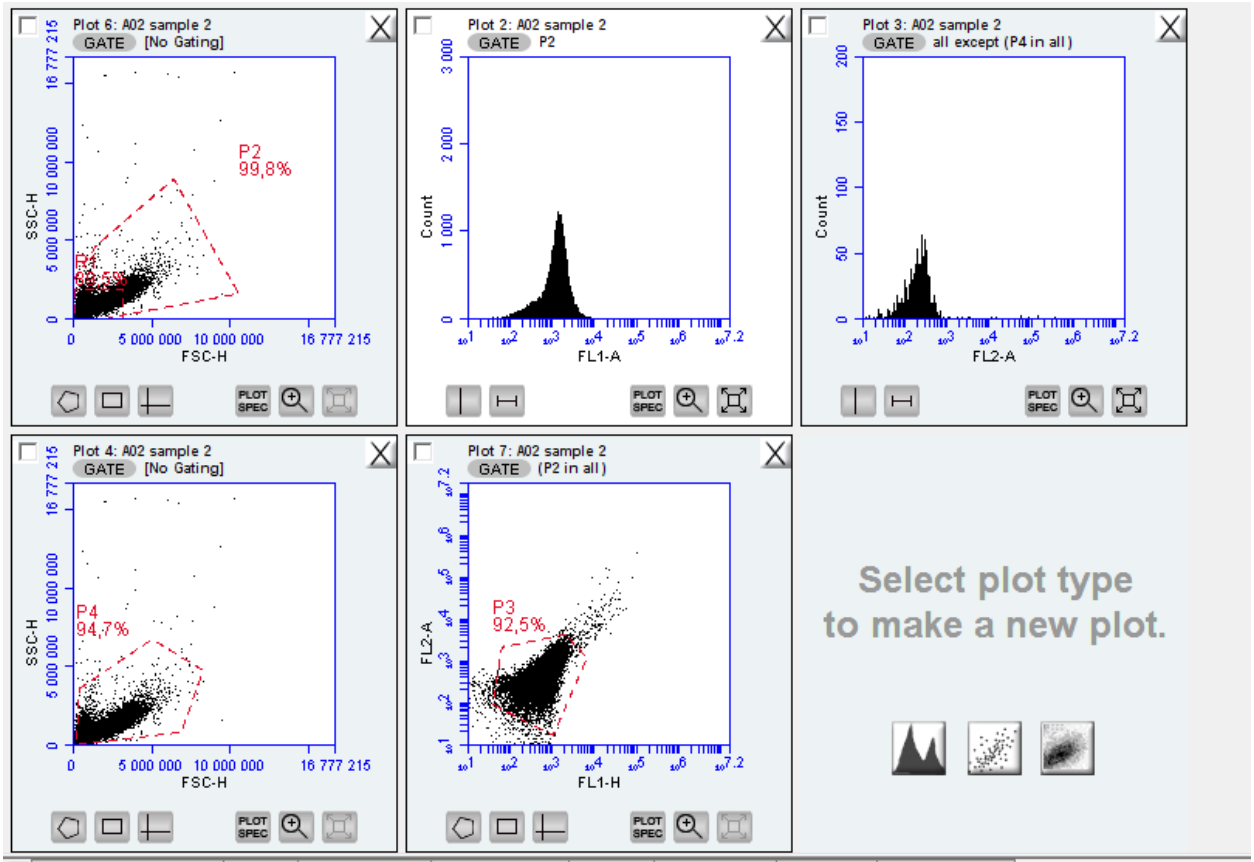
Plot 2: A01 sample 1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
This Plot	19 909	244	100,00%	99,54%	1 744,79	643,08%	

Plot 3: A01 sample 1 Gated on all except (P4 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 144	244	100,00%	5,72%	3 391,60	905,12%	

Plot 4: A01 sample 1	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-H	Mean SSC-H	CV FSC-H	CV SSC-H	Median FSC-H
All	20 000	244	100,00%	100,00%	1 707 171,94	854 531,29	68,87%	96,33%	
P4	18 856	244	94,28%	94,28%	1 785 990,89	856 405,54	62,41%	70,40%	

Figure 31. Sample Nr. 1. Treated with DAPI. Prepared according to Appendix A19.

Appendix B5



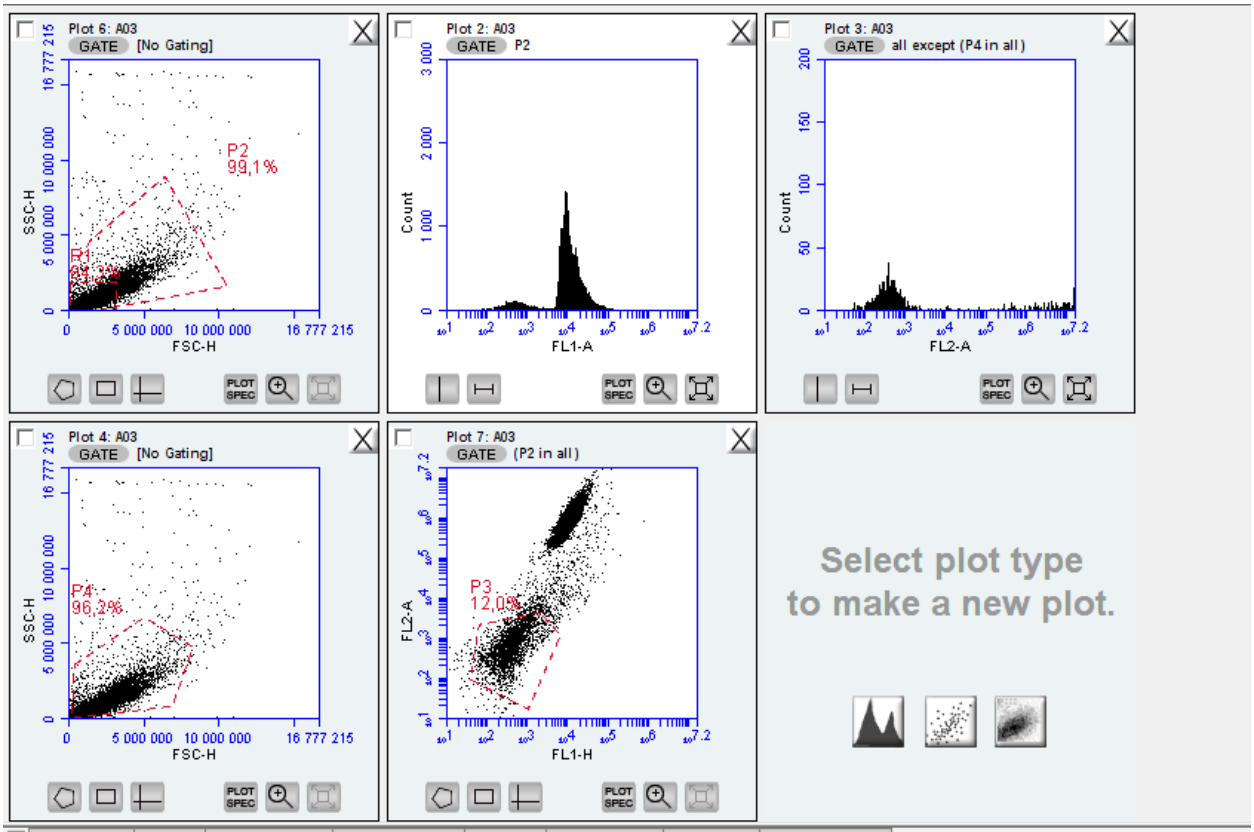
Plot 2: A02 sample 2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
This Plot	19 962	160	100,00%	99,81%	1 653,90	488,57%	

Plot 3: A02 sample 2 Gated on all except (P4 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 053	160	100,00%	5,27%	4 183,23	1 384,36%	

Plot 4: A02 sample 2	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-H	Mean SSC-H	CV FSC-H	CV SSC-H	Median FSC-H
All	20 000	160	100,00%	100,00%	1 734 519,00	908 975,36	65,18%	80,01%	
P4	18 947	160	94,74%	94,74%	1 814 704,64	930 336,61	59,38%	64,69%	

Figure 32. Sample Nr. 2. Treated with DAPI. Prepared according to Appendix A19.

Appendix B6



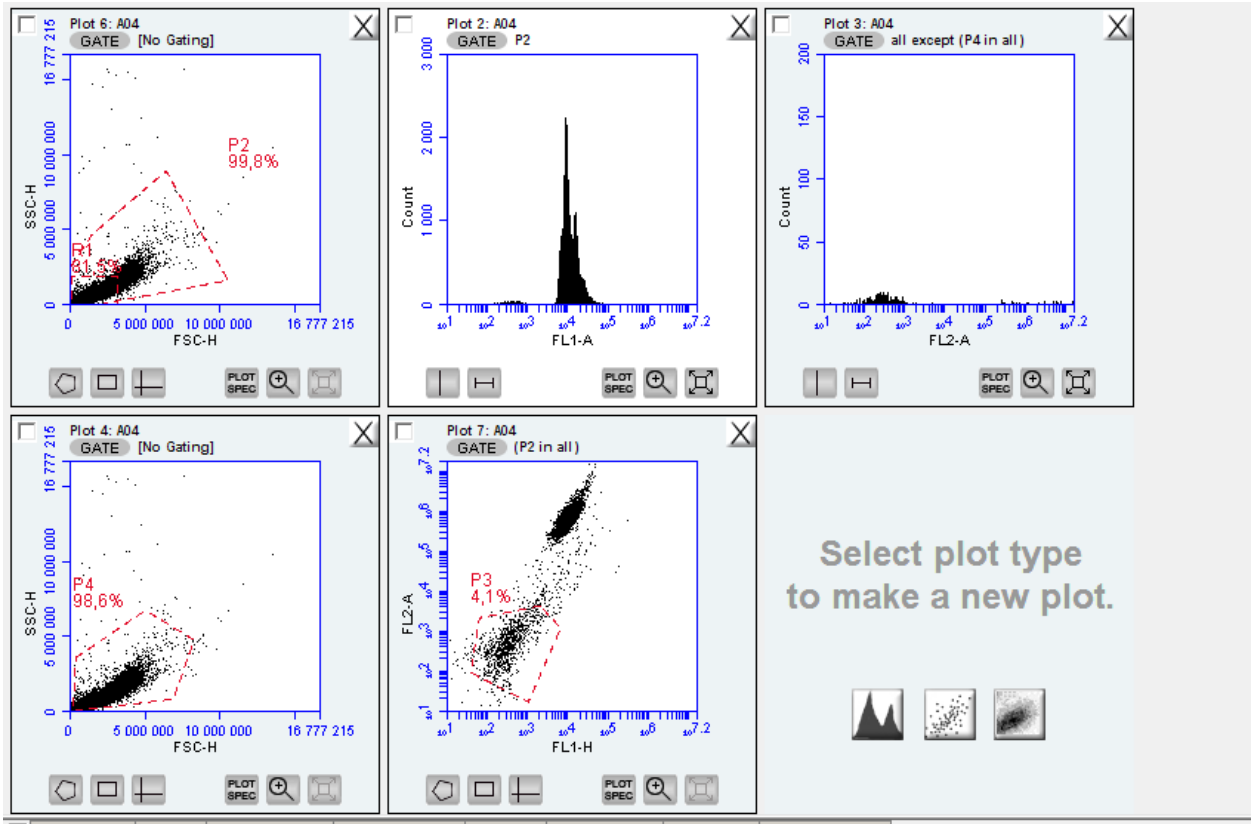
Plot 2: A03 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
This Plot	19 822	429	100,00%	99,11%	14 928,98	191,12%	

Plot 3: A03 Gated on all except (P4 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	757	429	100,00%	3,78%	1 417 357,79	258,56%	

Plot 4: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-H	Mean SSC-H	CV FSC-H	CV SSC-H	Median FSC-H	Median SSC-H
All	20 000	429	100,00%	100,00%	1 998 412,32	956 463,47	66,74%	115,33%		
P4	19 243	429	96,21%	96,21%	2 002 658,45	895 973,59	59,67%	75,35%		

Figure 33. Sample Nr. 3. Treated with PI. Prepared according to Appendix A20.

Appendix B7



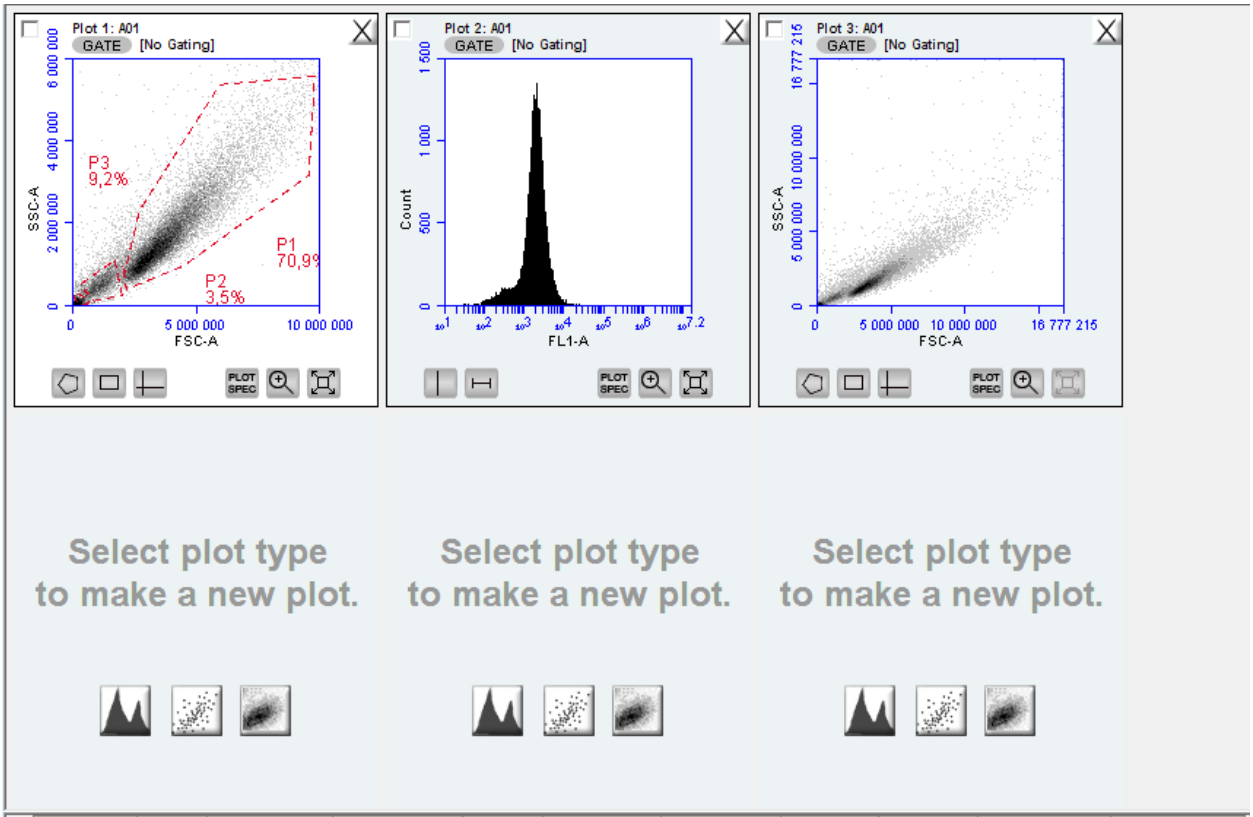
Plot 2: A04 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
This Plot	19 959	90	100,00%	99,80%	12 939,49	120,16%	

Plot 3: A04 Gated on all except (P4 in all)	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	280	90	100,00%	1,40%	764 331,80	326,37%	

Plot 4: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-H	Mean SSC-H	CV FSC-H	CV SSC-H	Median FSC-H	Median SSC-H
All	20 000	90	100,00%	100,00%	2 339 846,79	1 031 937,19	43,99%	69,03%		
P4	19 720	90	98,60%	98,60%	2 352 450,03	1 020 957,12	41,36%	55,12%		

Figure 34. Sample Nr. 4. Treated with PI. Prepared according to Appendix A20.

Appendix B8



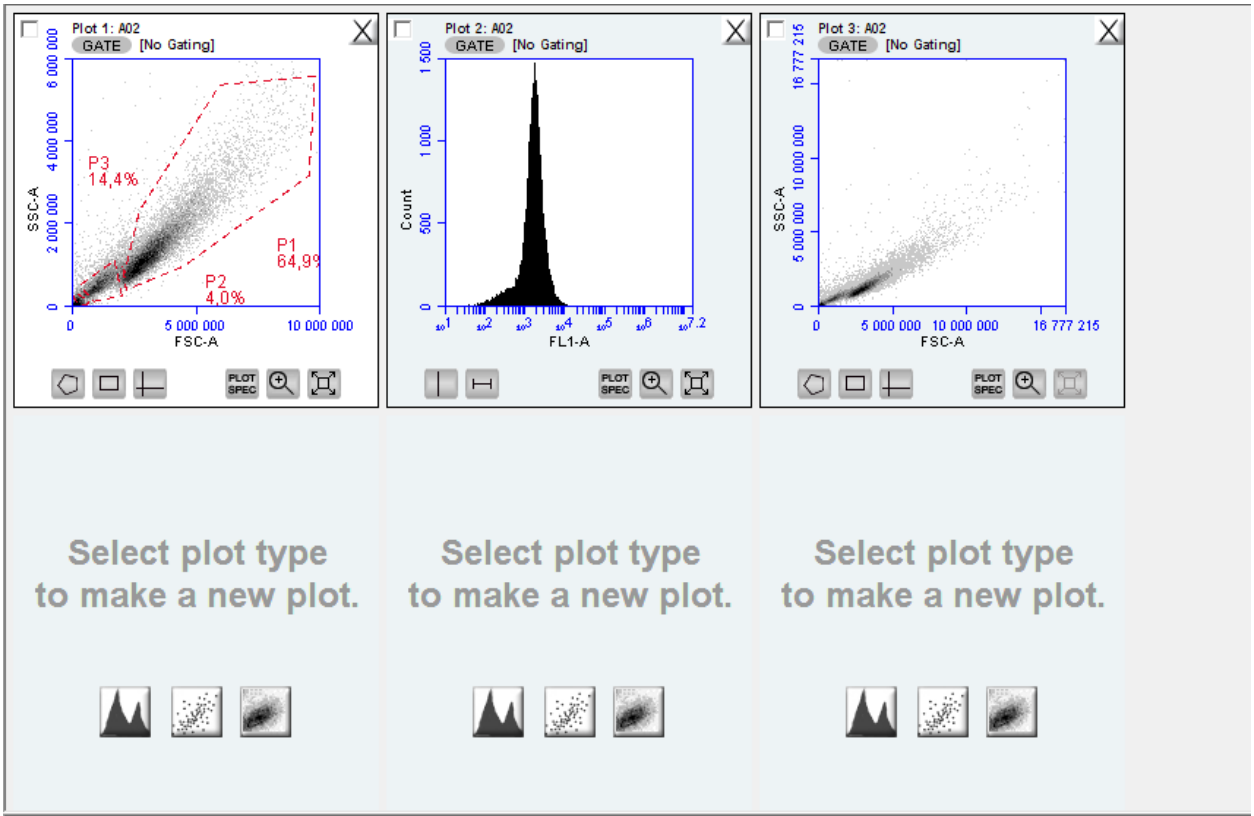
Plot 1: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	283	100,00%	100,00%	3 370 541,34	1 624 475,37	68,98%	97,80%		
P1	14 174	283	70,87%	70,87%	4 035 193,39	1 806 884,01	34,42%	49,94%		
P2	703	283	3,52%	3,52%	265 244,72	91 858,88	42,82%	60,24%		
P3	1 848	283	9,24%	9,24%	1 152 455,55	530 536,59	29,12%	35,29%		

Plot 2: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	283	100,00%	100,00%	3 084,96	1 063,66%	

Plot 3: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	283	100,00%	100,00%	3 370 541,34	1 624 475,37	68,98%	97,80%		

Figure 35. Sample Nr. 1. Treated with DAPI. Prepared according to Appendix A21.

Appendix B9



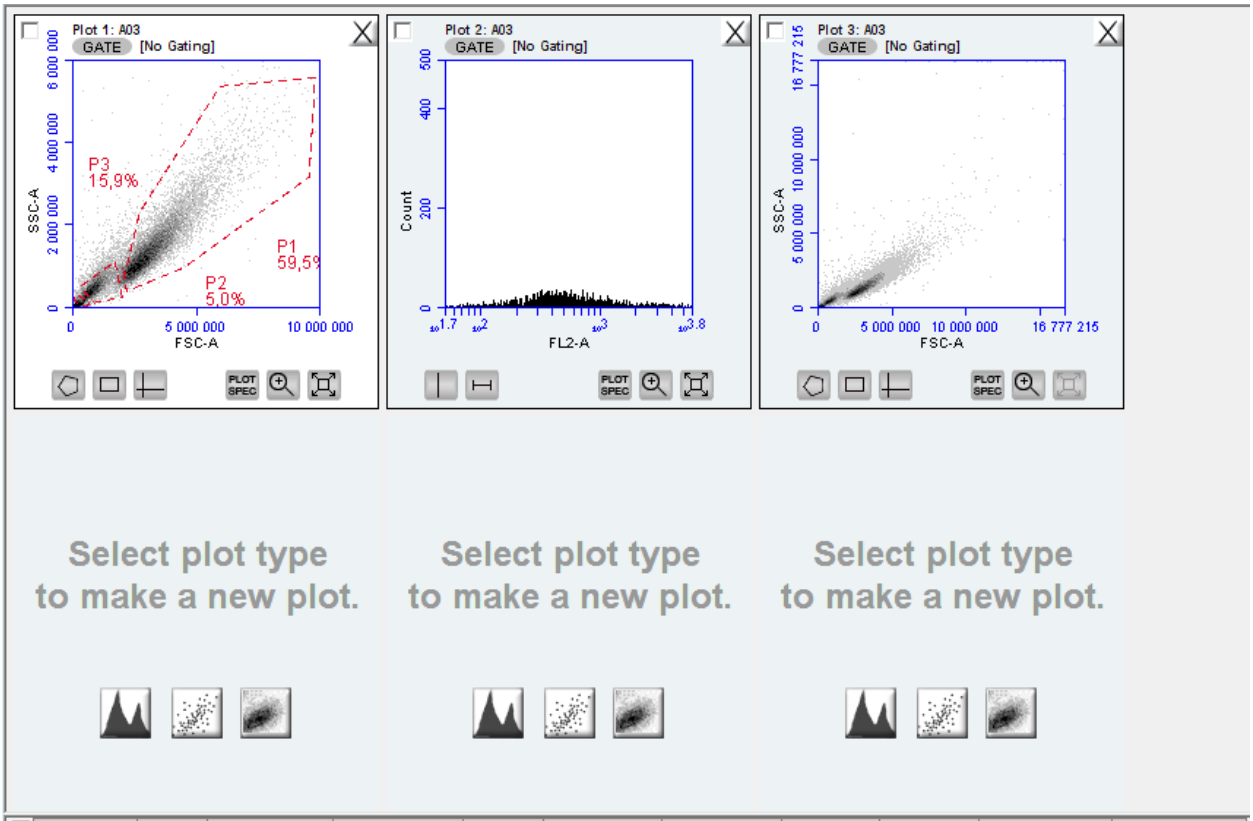
Plot 1: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	203	100,00%	100,00%	2 712 336,32	1 231 881,20	67,76%	90,13%		
P1	12 980	203	64,90%	64,90%	3 561 697,83	1 536 753,21	34,27%	50,99%		
P2	798	203	3,99%	3,99%	259 037,34	103 853,97	39,17%	56,71%		
P3	2 890	203	14,45%	14,45%	1 133 660,02	542 713,30	30,29%	32,30%		

Plot 2: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	203	100,00%	100,00%	2 089,23	288,40%	

Plot 3: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	203	100,00%	100,00%	2 712 336,32	1 231 881,20	67,76%	90,13%		

Figure 36. Sample Nr. 2. Treated with DAPI. Prepared according to Appendix A21.

Appendix B10



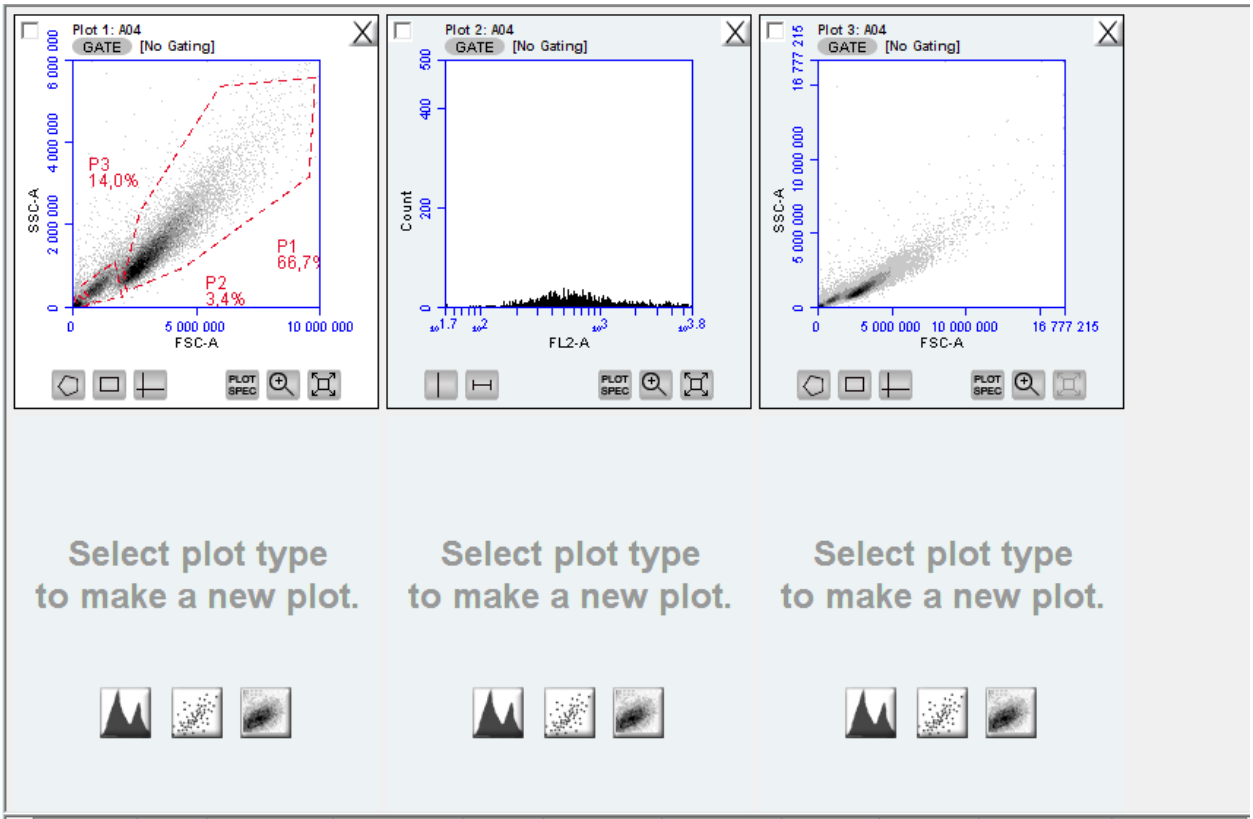
Plot 1: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	153	100,00%	100,00%	2 383 913,47	1 146 835,27	69,95%	93,19%		
P1	11 893	153	59,46%	59,46%	3 365 933,72	1 537 510,18	30,04%	45,81%		
P2	1 006	153	5,03%	5,03%	271 410,79	96 176,60	37,71%	56,44%		
P3	3 182	153	15,91%	15,91%	998 620,16	517 520,23	34,33%	33,93%		

Plot 2: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	153	100,00%	100,00%	462 524,27	116,06%	

Plot 3: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	153	100,00%	100,00%	2 383 913,47	1 146 835,27	69,95%	93,19%		

Figure 37. Sample Nr. 3. Treated with PI. Prepared according to Appendix A20.

Appendix B11



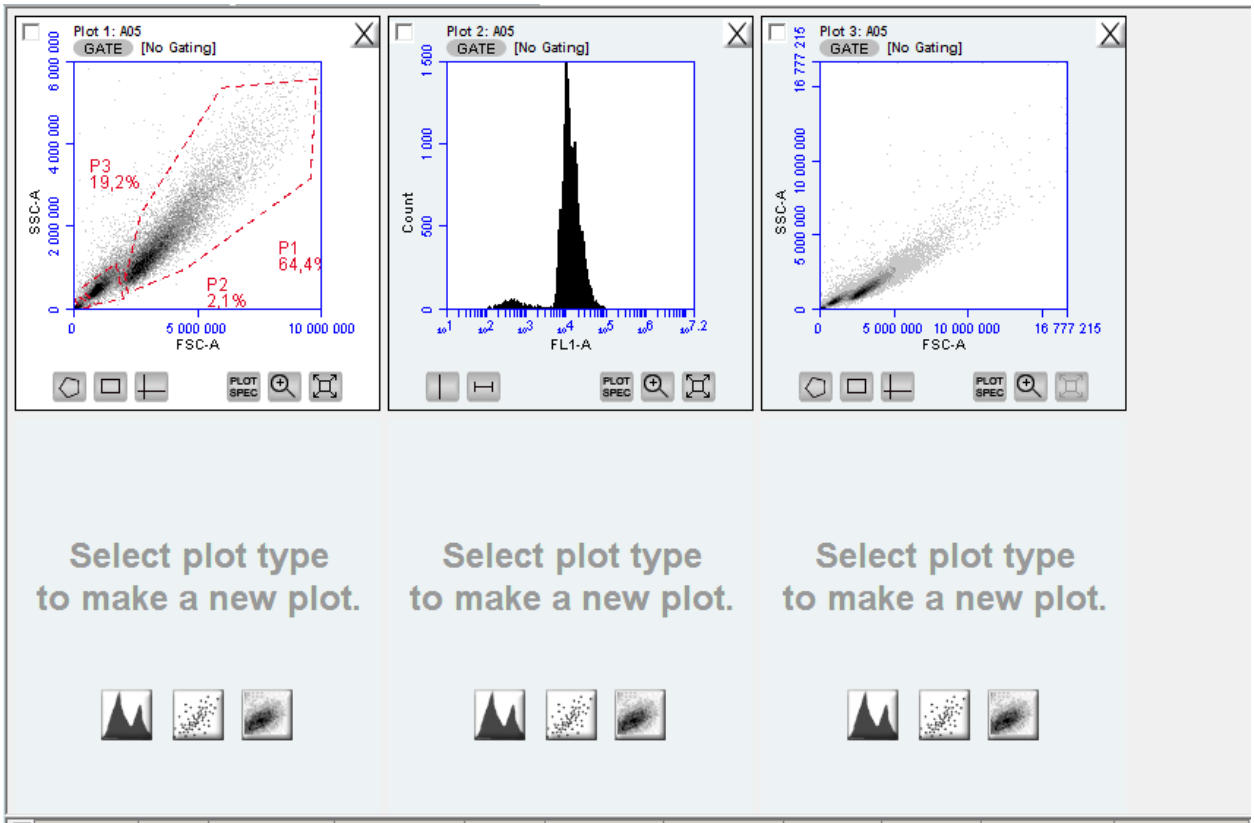
Plot 1: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	85	100,00%	100,00%	2 722 461,57	1 242 215,60	66,08%	88,84%		
P1	13 338	85	66,69%	66,69%	3 473 232,89	1 506 083,28	33,94%	50,04%		
P2	681	85	3,40%	3,40%	267 990,48	101 332,36	43,41%	55,34%		
P3	2 801	85	14,00%	14,00%	1 105 569,03	532 441,04	31,33%	33,61%		

Plot 2: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	85	100,00%	100,00%	475 510,47	107,25%	

Plot 3: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	85	100,00%	100,00%	2 722 461,57	1 242 215,60	66,08%	88,84%		

Figure 38. Sample Nr. 4. Treated with PI. Prepared according to Appendix A20.

Appendix B12



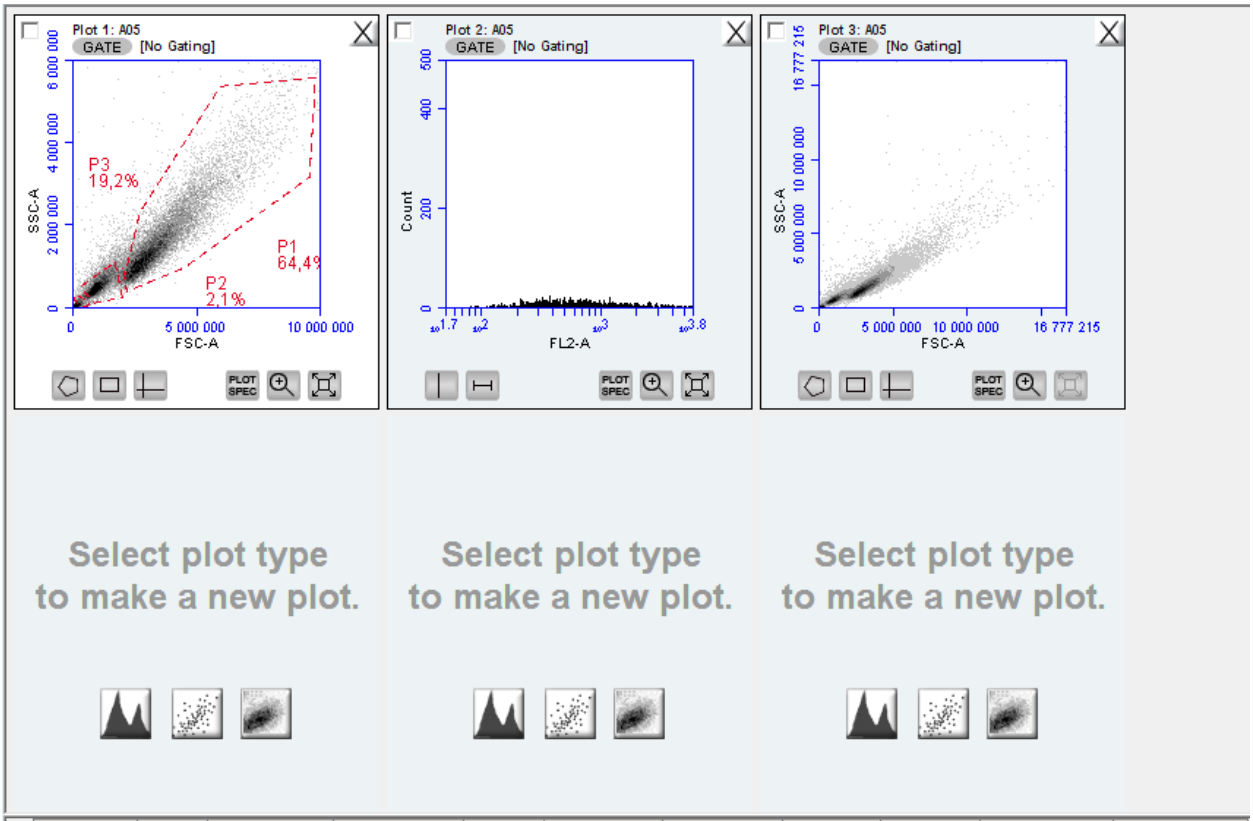
Plot 1: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	120	100,00%	100,00%	2 807 848,82	1 367 247,75	66,52%	86,92%		
P1	12 882	120	64,41%	64,41%	3 622 398,54	1 673 634,58	34,11%	49,79%		
P2	425	120	2,12%	2,12%	258 850,19	107 442,74	38,87%	50,90%		
P3	3 833	120	19,16%	19,16%	1 084 687,47	550 283,16	29,62%	32,79%		

Plot 2: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	120	100,00%	100,00%	14 876,78	86,00%	

Plot 3: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	120	100,00%	100,00%	2 807 848,82	1 367 247,75	66,52%	86,92%		

Figure 39. Sample Nr. 5. Treated with both DAPI & PI. DAPI shown. Prepared according to Appendix A22.

Appendix B13



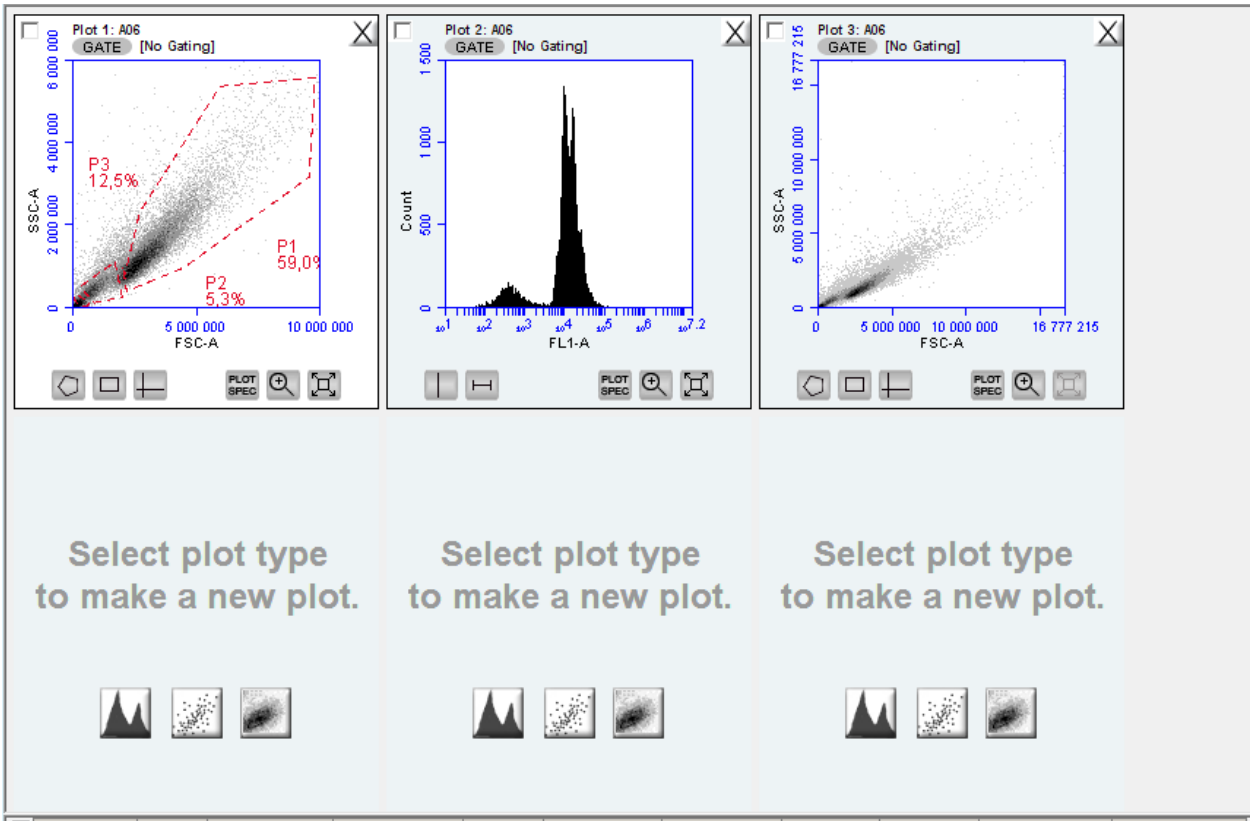
Plot 1: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	120	100,00%	100,00%	2 807 848,82	1 367 247,75	66,52%	86,92%		
P1	12 882	120	64,41%	64,41%	3 622 398,54	1 673 634,58	34,11%	49,79%		
P2	425	120	2,12%	2,12%	258 850,19	107 442,74	38,87%	50,90%		
P3	3 833	120	19,16%	19,16%	1 084 687,47	550 283,16	29,62%	32,79%		

Plot 2: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	120	100,00%	100,00%	401 129,71	82,93%	

Plot 3: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	120	100,00%	100,00%	2 807 848,82	1 367 247,75	66,52%	86,92%		

Figure 40. Sample Nr. 5. Treated with both DAPI & PI. PI shown. Prepared according to Appendix A22.

Appendix B14



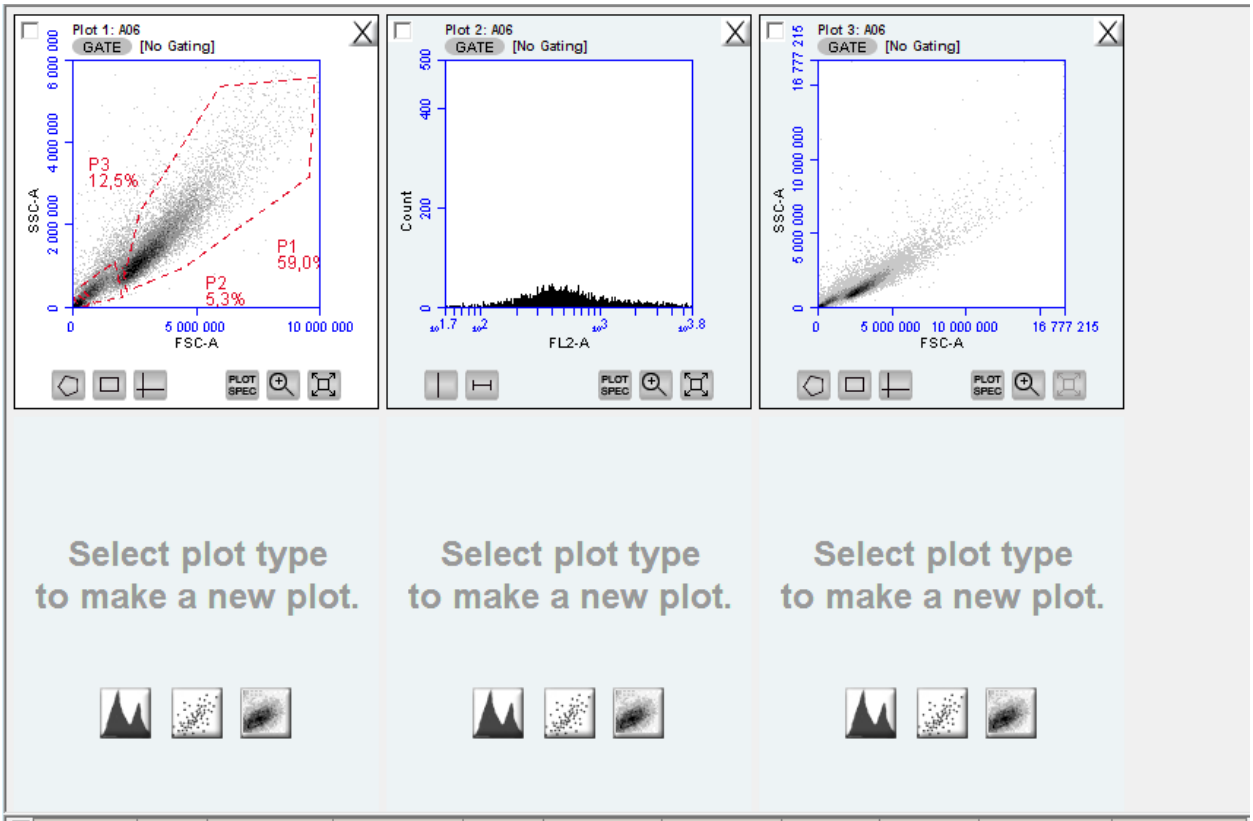
Plot 1: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	273	100,00%	100,00%	2 518 372,73	1 240 240,36	73,41%	92,38%		
P1	11 801	273	59,00%	59,00%	3 475 694,56	1 609 901,68	33,98%	49,04%		
P2	1 054	273	5,27%	5,27%	266 296,40	105 787,41	41,19%	52,09%		
P3	2 505	273	12,52%	12,52%	1 073 740,04	529 813,29	35,36%	33,59%		

Plot 2: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	273	100,00%	100,00%	14 211,64	152,08%	

Plot 3: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	273	100,00%	100,00%	2 518 372,73	1 240 240,36	73,41%	92,38%		

Figure 41. Sample Nr. 6. Treated with both DAPI & PI. DAPI shown. Prepared according to Appendix A22.

Appendix B15



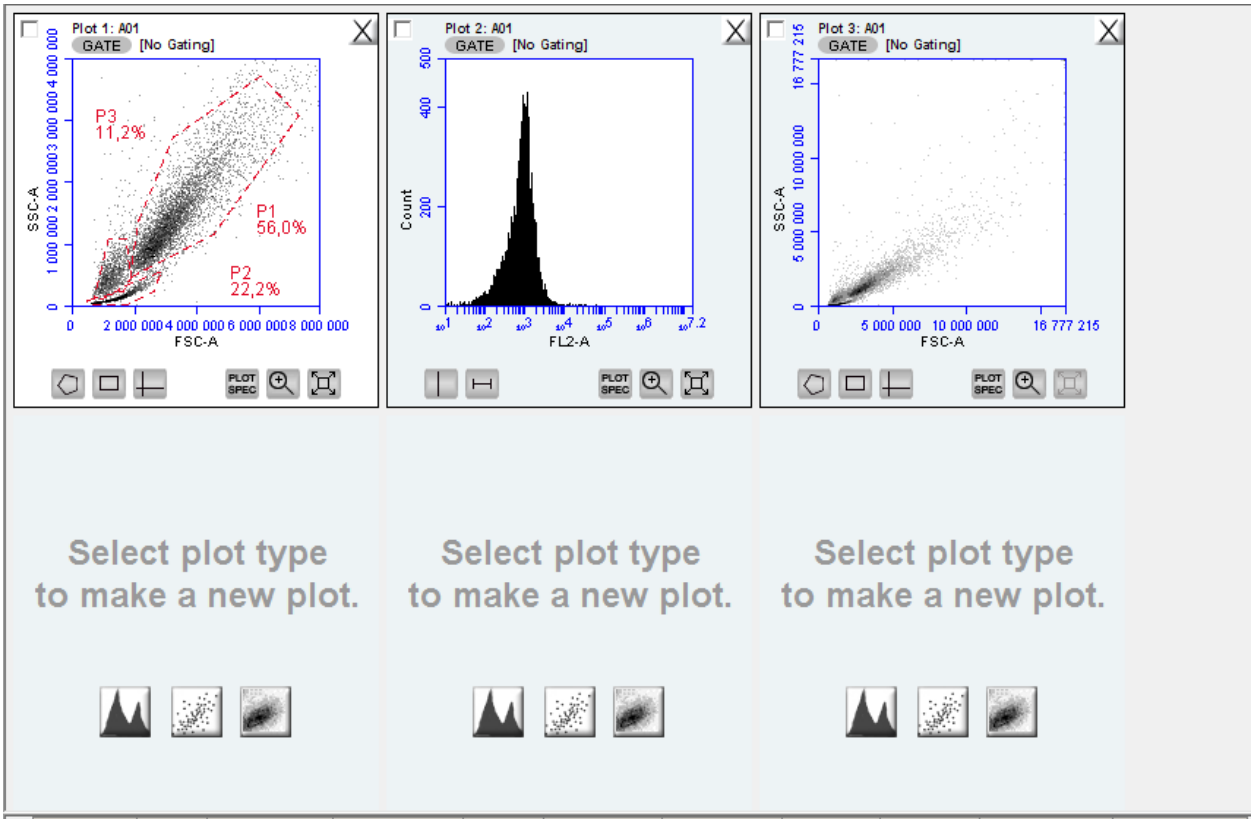
Plot 1: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	273	100,00%	100,00%	2 518 372,73	1 240 240,36	73,41%	92,38%		
P1	11 801	273	59,00%	59,00%	3 475 694,56	1 609 901,68	33,98%	49,04%		
P2	1 054	273	5,27%	5,27%	266 296,40	105 787,41	41,19%	52,09%		
P3	2 505	273	12,52%	12,52%	1 073 740,04	529 813,29	35,36%	33,59%		

Plot 2: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	273	100,00%	100,00%	384 857,52	97,25%	

Plot 3: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	273	100,00%	100,00%	2 518 372,73	1 240 240,36	73,41%	92,38%		

Figure 42. Sample Nr. 6 Treated with both DAPI & PI. PI shown. Prepared according to Appendix A22.

Appendix B16



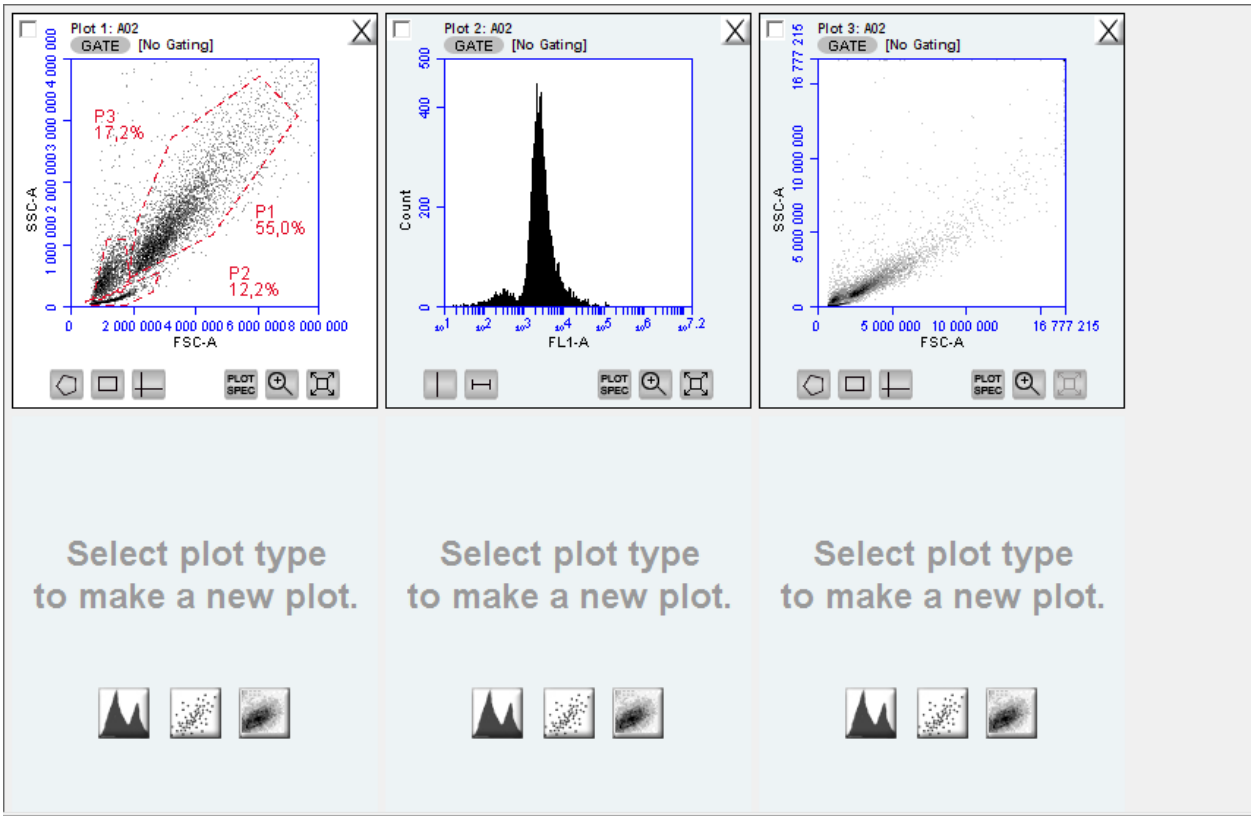
Plot 1: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	7 838	402	100,00%	100,00%	2 972 846,70	1 353 898,47	72,54%	127,15%		
P1	4 386	402	55,96%	55,96%	3 392 545,90	1 491 740,55	29,13%	41,64%		
P2	1 738	402	22,17%	22,17%	1 198 143,92	99 800,20	39,29%	83,47%		
P3	875	402	11,16%	11,16%	1 247 737,88	495 268,10	20,81%	40,45%		

Plot 2: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	7 838	402	100,00%	100,00%	1 931,09	1 006,08%	

Plot 3: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	7 838	402	100,00%	100,00%	2 972 846,70	1 353 898,47	72,54%	127,15%		

Figure 43. Sample Nr. 1. Treated with DAPI. Prepared according to Appendix A23.

Appendix B17



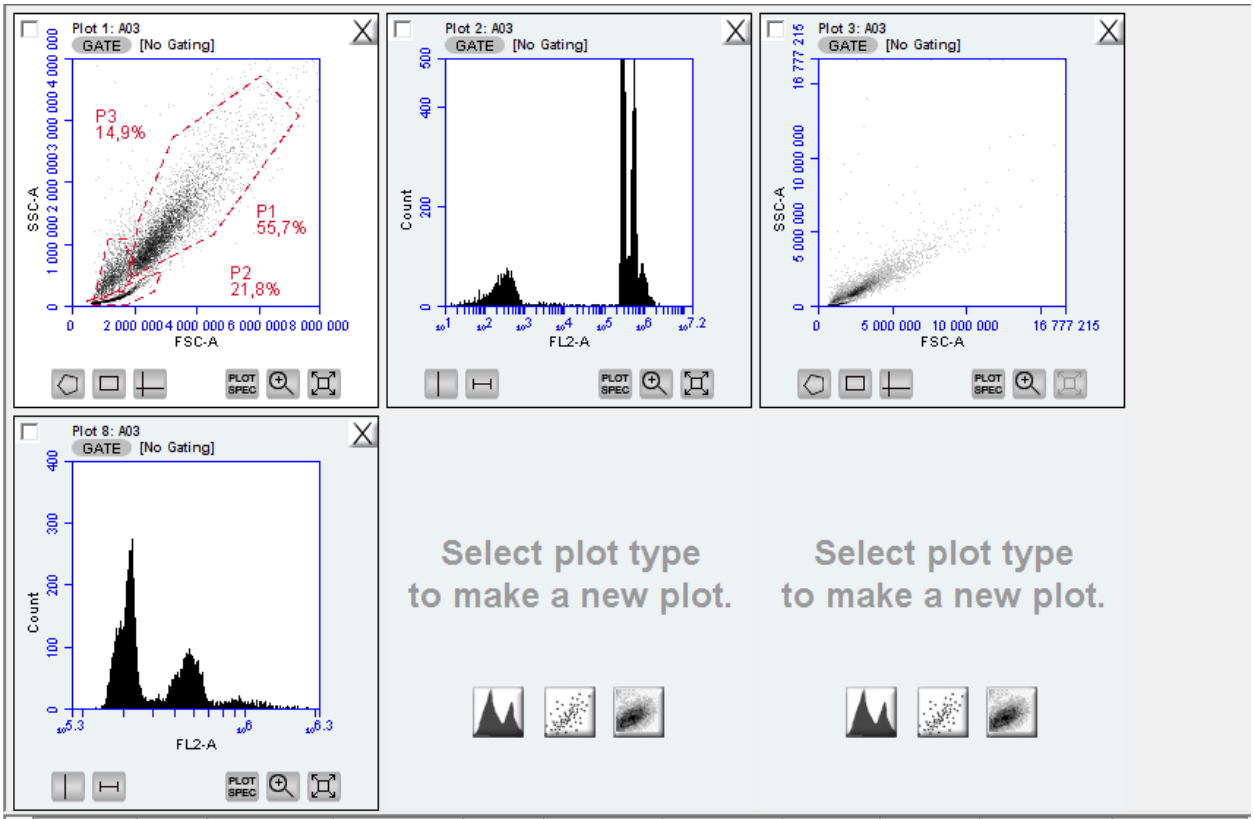
Plot 1: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	7 164	358	100,00%	100,00%	3 228 386,25	1 591 335,31	83,37%	143,04%		
P1	3 940	358	55,00%	55,00%	3 402 520,30	1 424 613,21	30,41%	44,30%		
P2	871	358	12,16%	12,16%	1 227 235,93	104 157,54	38,75%	79,89%		
P3	1 235	358	17,24%	17,24%	1 214 565,72	490 231,77	21,85%	42,29%		

Plot 2: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	7 164	358	100,00%	100,00%	5 388,85	712,33%	

Plot 3: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	7 164	358	100,00%	100,00%	3 228 386,25	1 591 335,31	83,37%	143,04%		

Figure 44. Sample Nr. 2. Treated with DAPI. Prepared according to Appendix A23.

Appendix B18



Plot 1: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	8 028	296	100,00%	100,00%	2 484 399,55	1 007 223,79	61,16%	107,03%		
P1	4 471	296	55,69%	55,69%	3 119 364,17	1 304 224,72	29,02%	42,61%		
P2	1 753	296	21,84%	21,84%	1 162 208,66	98 347,75	36,72%	76,30%		
P3	1 200	296	14,95%	14,95%	1 300 842,31	492 479,79	20,87%	41,44%		

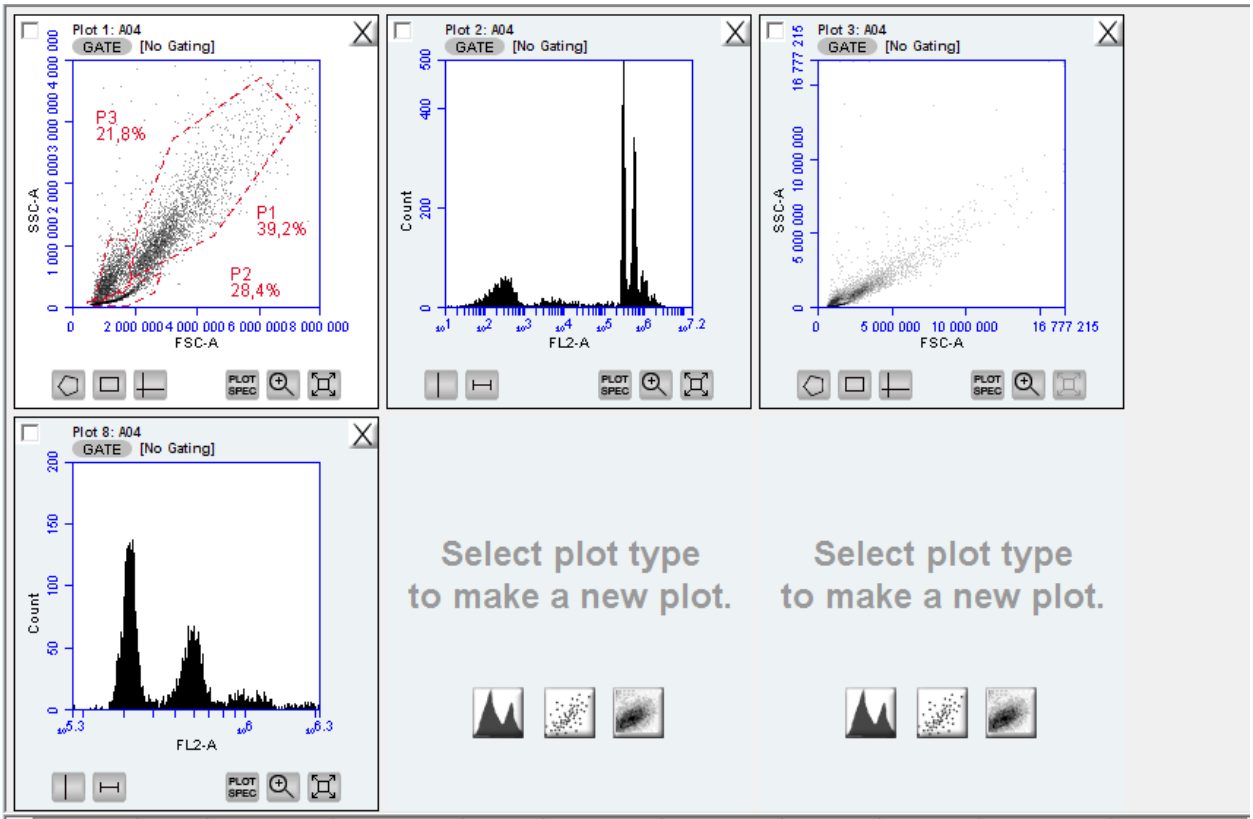
Plot 2: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	8 028	296	100,00%	100,00%	356 895,73	124,03%	

Plot 3: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	8 028	296	100,00%	100,00%	2 484 399,55	1 007 223,79	61,16%	107,03%		

Plot 8: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	8 028	296	100,00%	100,00%	356 895,73	124,03%	

Figure 45. Sample Nr. 3. Treated with PI. Prepared according to Appendix A24.

Appendix B19



Plot 1: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5 441	150	100,00%	100,00%	2 342 098,90	942 140,56	79,99%	142,03%		
P1	2 132	150	39,18%	39,18%	3 269 052,48	1 345 450,39	30,24%	44,92%		
P2	1 544	150	28,38%	28,38%	1 179 353,11	105 802,25	37,74%	78,85%		
P3	1 185	150	21,78%	21,78%	1 199 109,50	449 294,64	22,47%	46,53%		

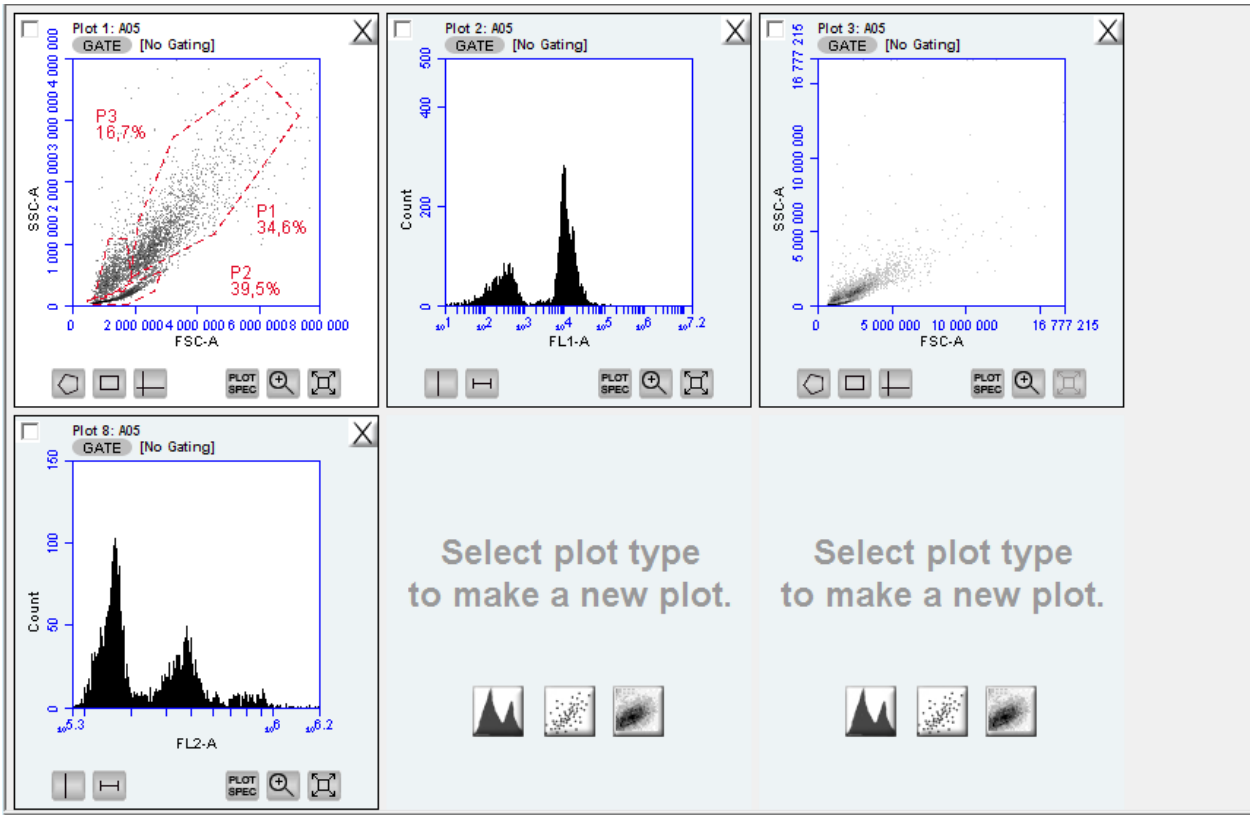
Plot 2: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	5 441	150	100,00%	100,00%	358 027,25	136,96%	

Plot 3: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5 441	150	100,00%	100,00%	2 342 098,90	942 140,56	79,99%	142,03%		

Plot 8: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	5 441	150	100,00%	100,00%	358 027,25	136,96%	

Figure 46. Sample Nr. 4. Treated with PI. Prepared according to Appendix A24.

Appendix B20



Plot 1: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5 327	335	100,00%	100,00%	2 063 314,72	746 225,20	70,49%	148,95%		
P1	1 841	335	34,56%	34,56%	3 066 115,94	1 255 616,04	29,56%	43,47%		
P2	2 105	335	39,52%	39,52%	1 198 503,13	102 342,00	39,36%	82,61%		
P3	890	335	16,71%	16,71%	1 271 203,94	491 452,39	21,05%	40,77%		

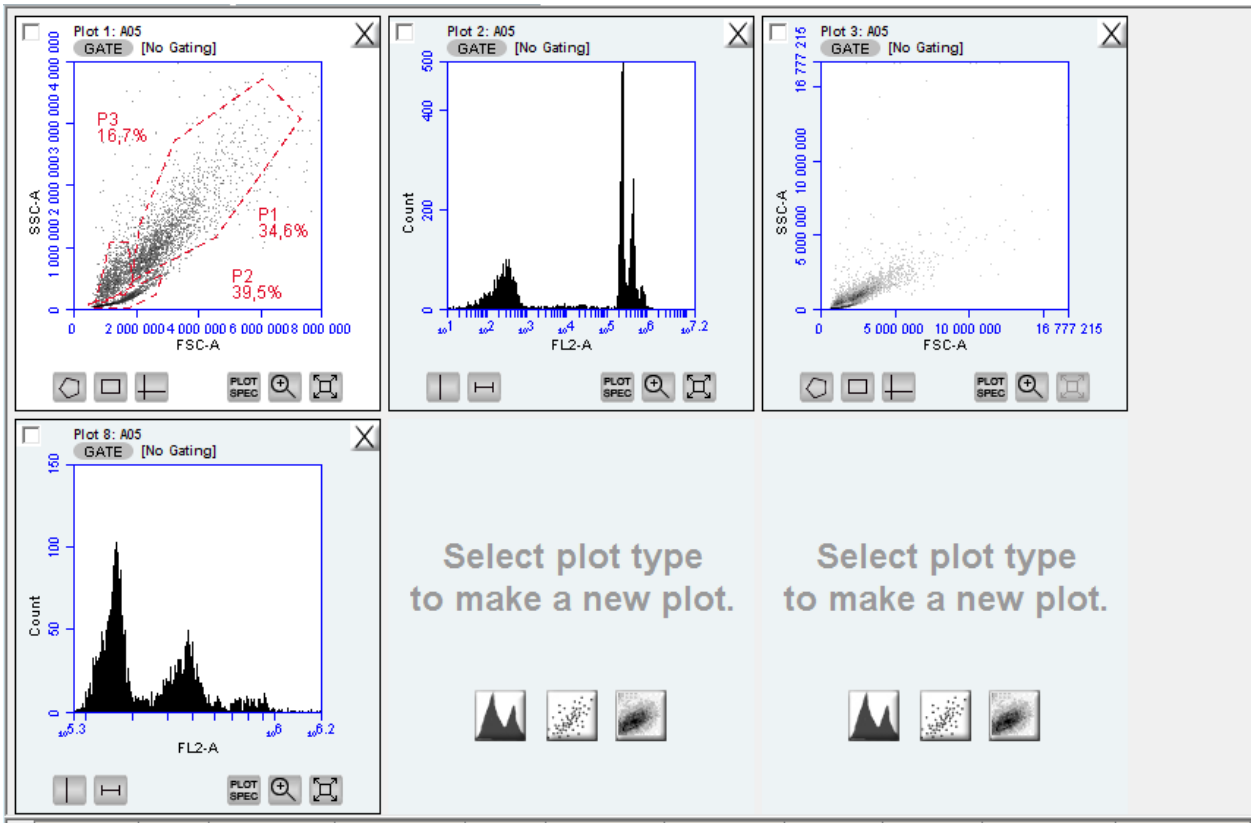
Plot 2: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	5 327	335	100,00%	100,00%	8 703,17	260,55%	

Plot 3: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5 327	335	100,00%	100,00%	2 063 314,72	746 225,20	70,49%	148,95%		

Plot 8: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	5 327	335	100,00%	100,00%	196 141,77	120,86%	

Figure 47. Sample Nr. 5. Treated with DAPI&PI. DAPI shown. Prepared according to Appendix A25.

Appendix B21



Plot 1: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5 327	335	100,00%	100,00%	2 063 314,72	746 225,20	70,49%	148,95%		
P1	1 841	335	34,56%	34,56%	3 066 115,94	1 255 616,04	29,56%	43,47%		
P2	2 105	335	39,52%	39,52%	1 198 503,13	102 342,00	39,36%	82,61%		
P3	890	335	16,71%	16,71%	1 271 203,94	491 452,39	21,05%	40,77%		

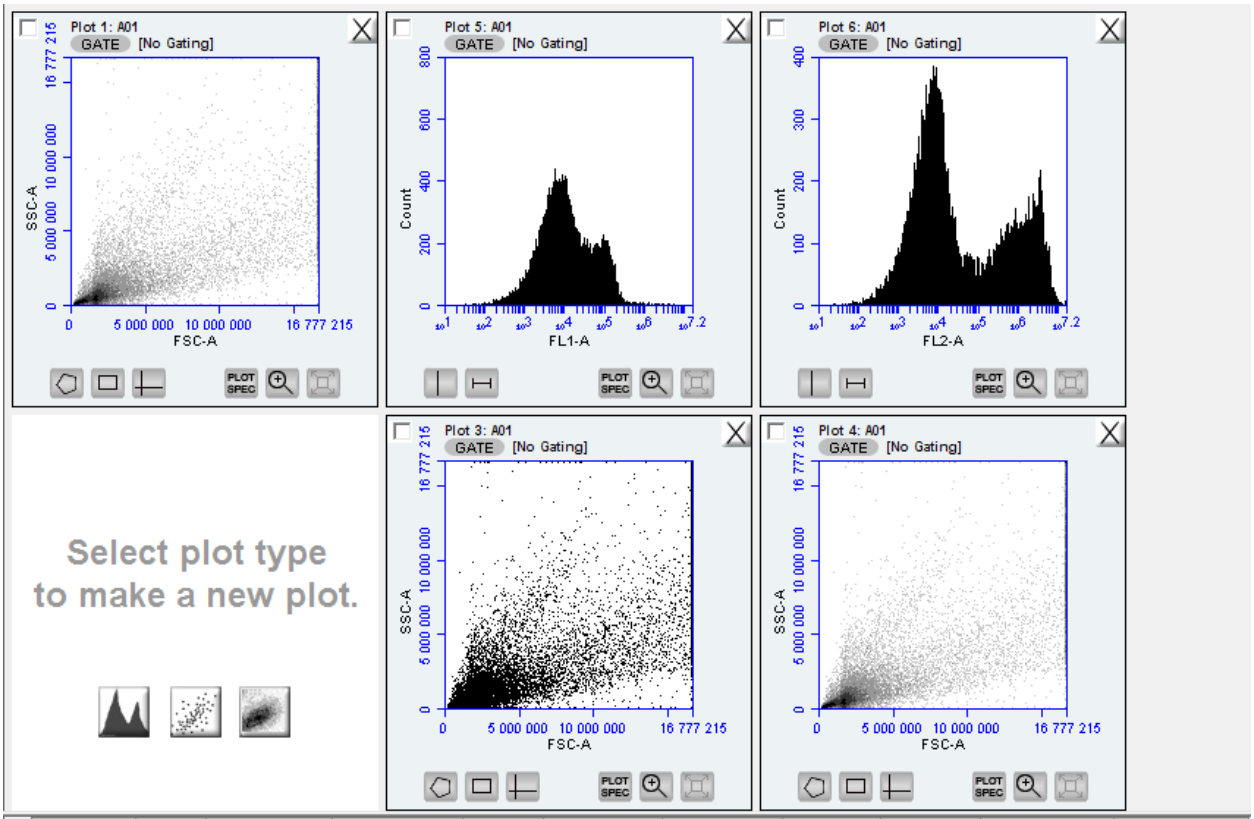
Plot 2: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	5 327	335	100,00%	100,00%	196 141,77	120,86%	

Plot 3: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	5 327	335	100,00%	100,00%	2 063 314,72	746 225,20	70,49%	148,95%		

Plot 8: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	5 327	335	100,00%	100,00%	196 141,77	120,86%	

Figure 48. Sample Nr. 5. Treated with DAPI&PI. PI shown. Prepared according to Appendix A25.

Appendix B22



Plot 3: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	228	100,00%	100,00%	4 956 041,37	2 788 546,09	111,14%	149,92%		

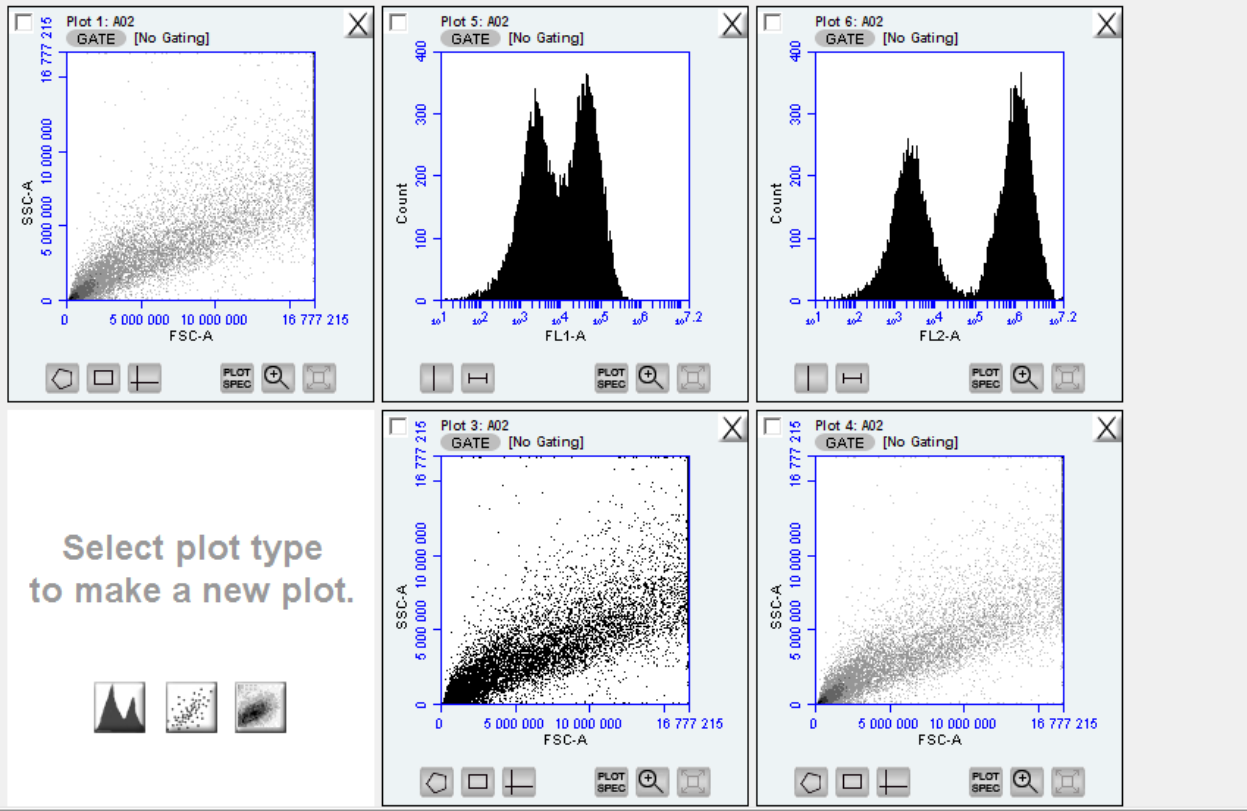
Plot 4: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	228	100,00%	100,00%	4 956 041,37	2 788 546,09	111,14%	149,92%		

Plot 5: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	228	100,00%	100,00%	48 355,64	594,40%	

Plot 6: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	228	100,00%	100,00%	621 530,19	233,37%	

Figure 49. Sample Nr. 1. Stained with DAPI & PI. Prepared according to Appendix A25.

Appendix B23



Plot 3: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	80	100,00%	100,00%	4 799 248,59	3 004 157,19	122,30%	143,30%		

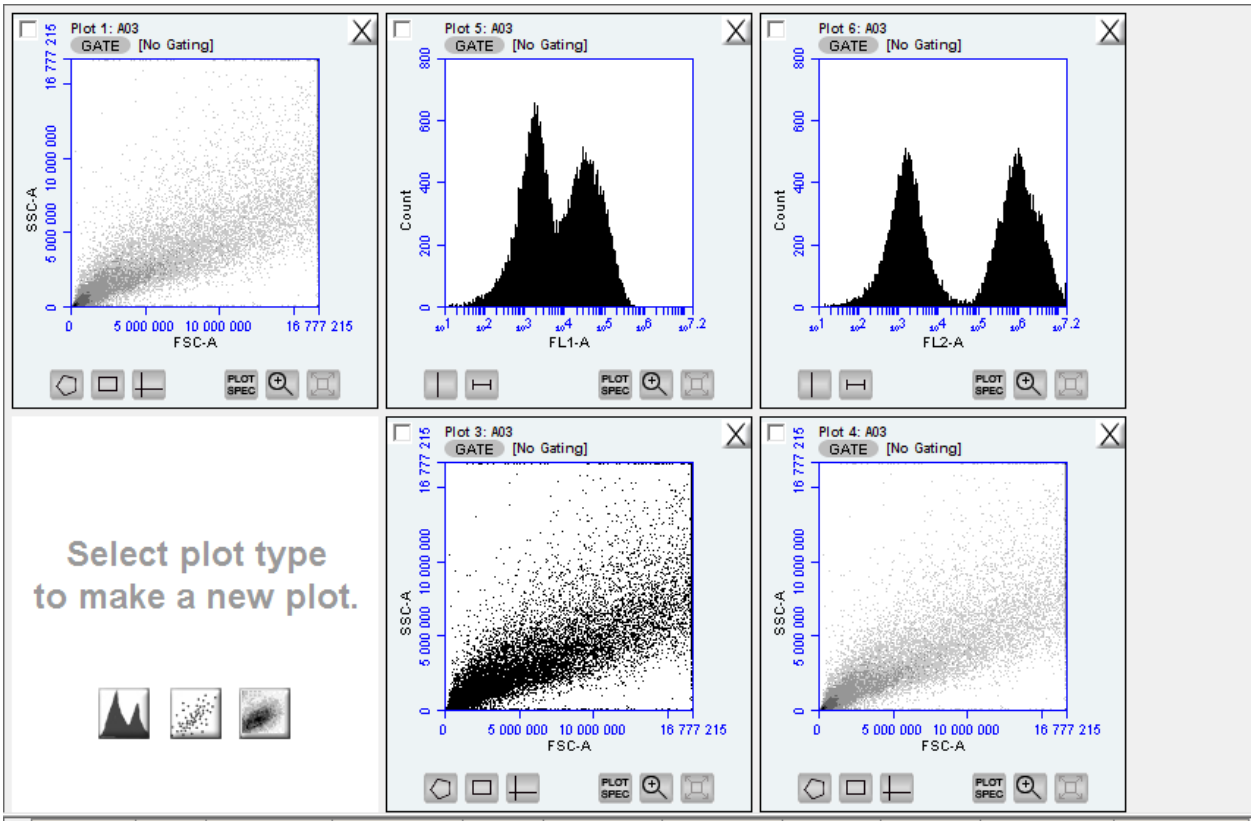
Plot 4: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	80	100,00%	100,00%	4 799 248,59	3 004 157,19	122,30%	143,30%		

Plot 5: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	80	100,00%	100,00%	30 195,79	203,44%	

Plot 6: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	80	100,00%	100,00%	742 752,12	179,37%	

Figure 50. Sample Nr. 2. Stained with DAPI & PI. Prepared according to Appendix A25.

Appendix B24



Plot 3: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	36 067	176	100,00%	100,00%	4 229 375,57	2 795 524,32	138,03%	162,93%		

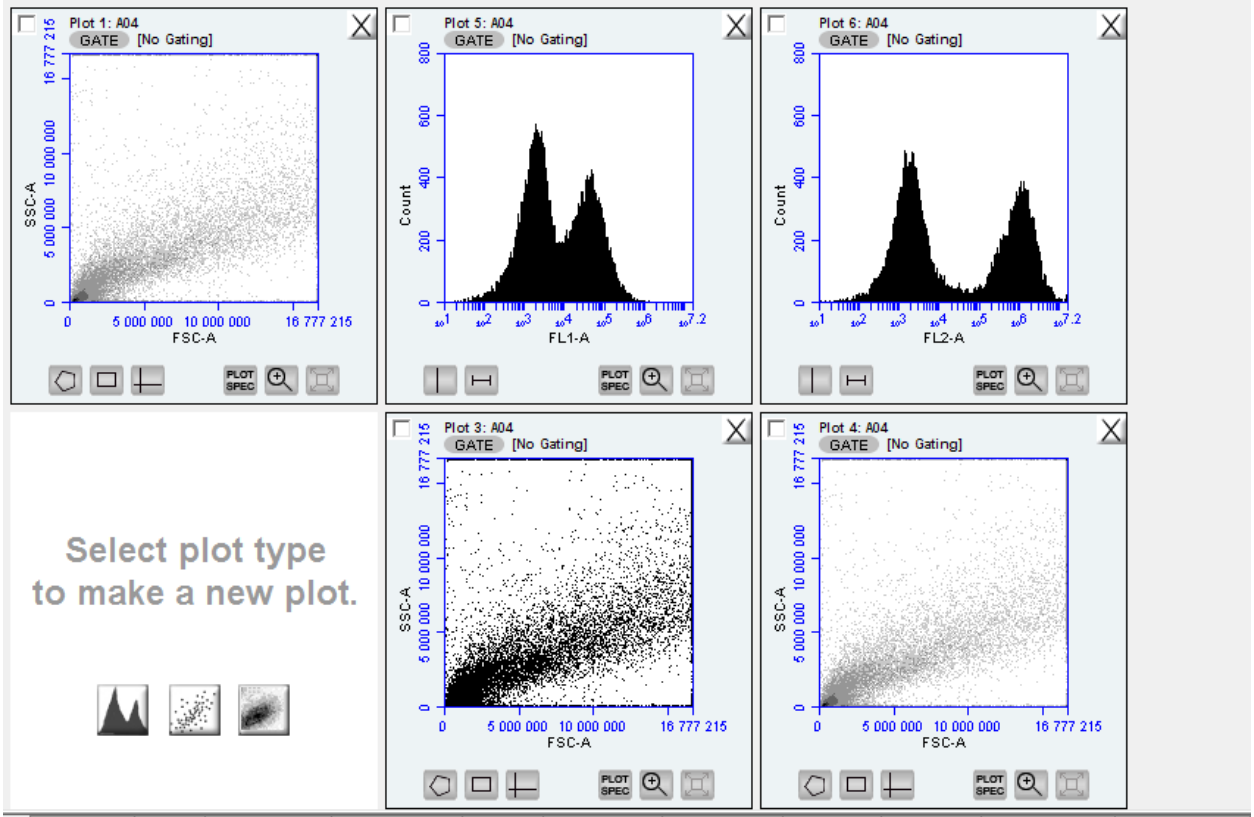
Plot 4: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	36 067	176	100,00%	100,00%	4 229 375,57	2 795 524,32	138,03%	162,93%		

Plot 5: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	36 067	176	100,00%	100,00%	28 392,34	318,88%	

Plot 6: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	36 067	176	100,00%	100,00%	903 208,72	217,54%	

Figure 51. Sample Nr. 3. Stained with DAPI & PI. Prepared according to Appendix A25.

Appendix B25



Plot 3: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	30 000	168	100,00%	100,00%	4 451 666,93	2 986 622,27	136,12%	166,67%		

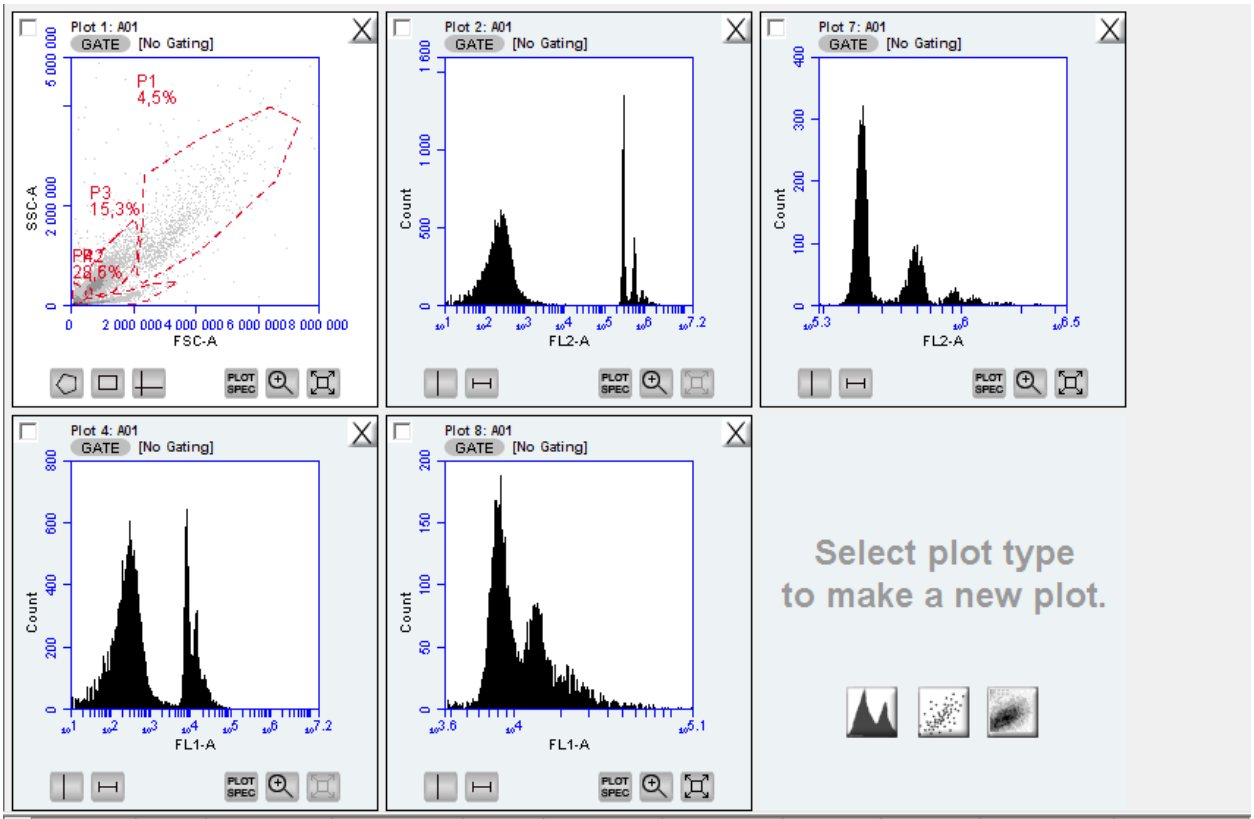
Plot 4: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	30 000	168	100,00%	100,00%	4 451 666,93	2 986 622,27	136,12%	166,67%		

Plot 5: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	30 000	168	100,00%	100,00%	28 816,08	289,60%	

Plot 6: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	30 000	168	100,00%	100,00%	527 192,17	237,63%	

Figure 52. Sample Nr. 4. Stained with DAPI & PI. Prepared according to Appendix A25.

Appendix B26



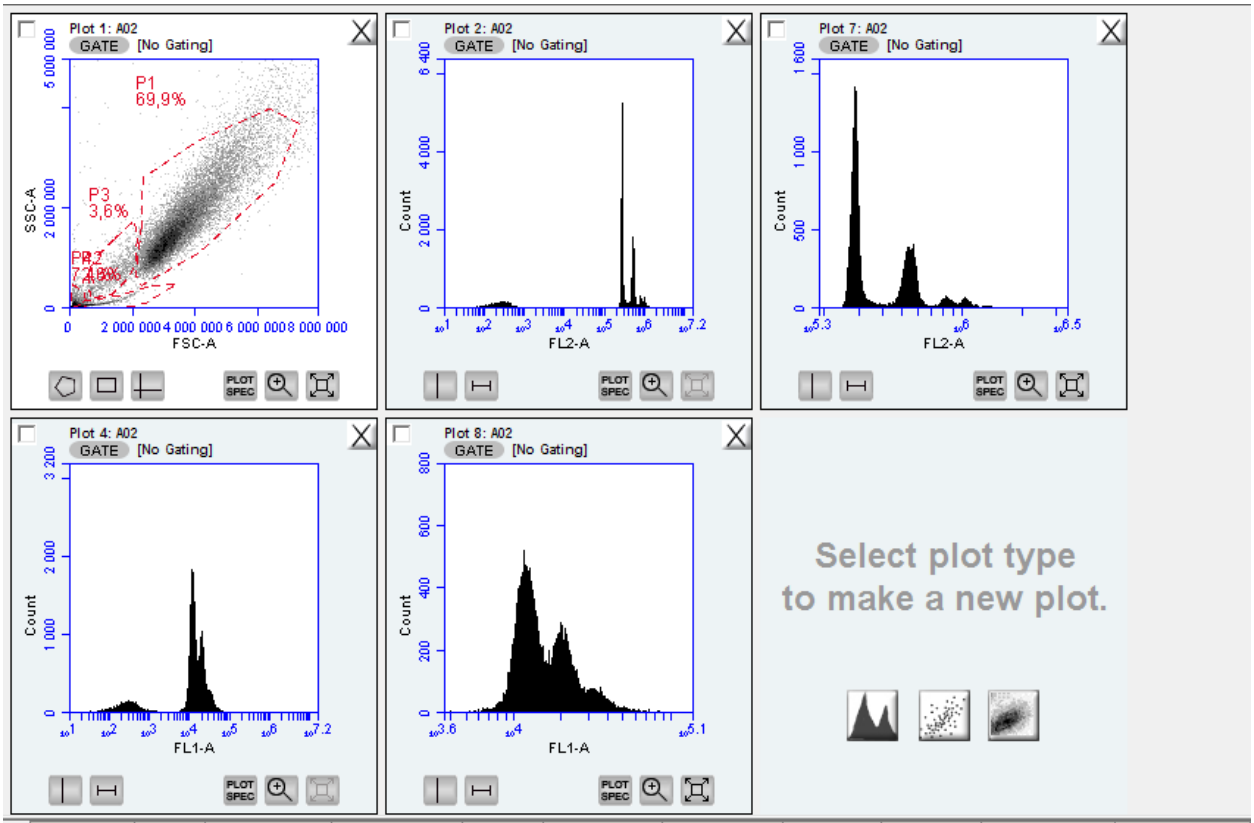
Plot 1: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	104	100,00%	100,00%	571 087,05	237 179,00	175,03%	289,08%		
P2	1 730	104	8,65%	8,65%	937 045,00	69 787,41	53,03%	84,53%		
P3	3 051	104	15,25%	15,25%	1 045 151,91	563 438,74	33,58%	44,05%		
P1	894	104	4,47%	4,47%	3 440 687,58	1 626 908,71	27,84%	40,10%		
P4	4 501	104	22,50%	22,50%	179 093,19	44 050,00	38,19%	133,10%		

Plot 2: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	104	100,00%	100,00%	118 016,53	256,47%	

Plot 4: A01	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	104	100,00%	100,00%	3 793,43	451,08%	

Figure 53. A-series sample Nr. 1. Stained with DAPI & PI. Prepared according to Appendix A26.

Appendix B27



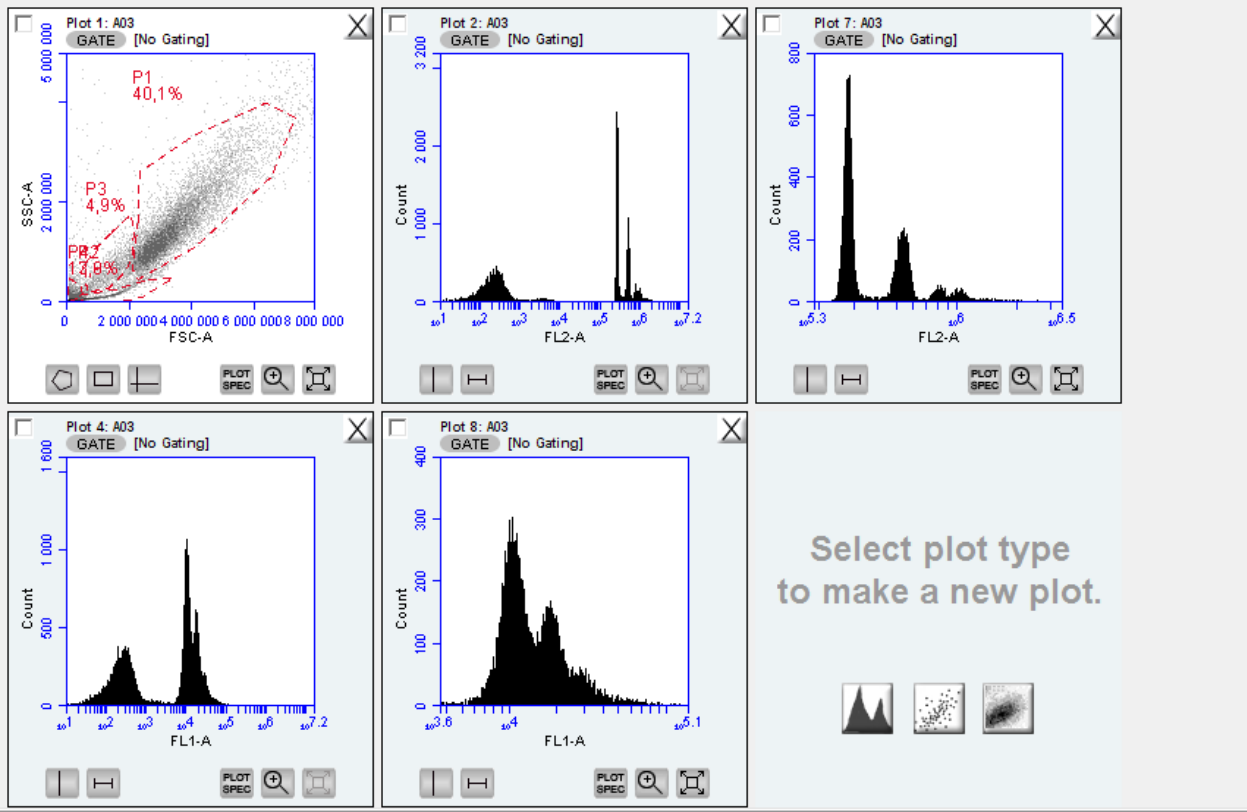
Plot 1: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	127	100,00%	100,00%	3 071 533,45	1 526 095,54	66,47%	89,66%		
P2	530	127	2,65%	2,65%	934 985,86	77 251,67	48,67%	73,34%		
P3	714	127	3,57%	3,57%	1 446 007,34	734 943,60	29,74%	37,60%		
P1	13 976	127	69,88%	69,88%	3 679 380,02	1 749 987,96	25,47%	38,11%		
P4	1 487	127	7,44%	7,44%	178 184,71	61 666,01	41,05%	115,49%		

Plot 2: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	127	100,00%	100,00%	354 674,29	109,72%	

Plot 4: A02	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	127	100,00%	100,00%	14 353,97	106,89%	

Figure 54. A-series sample Nr. 2. Stained with DAPI & PI. Prepared according to Appendix A27.

Appendix B28



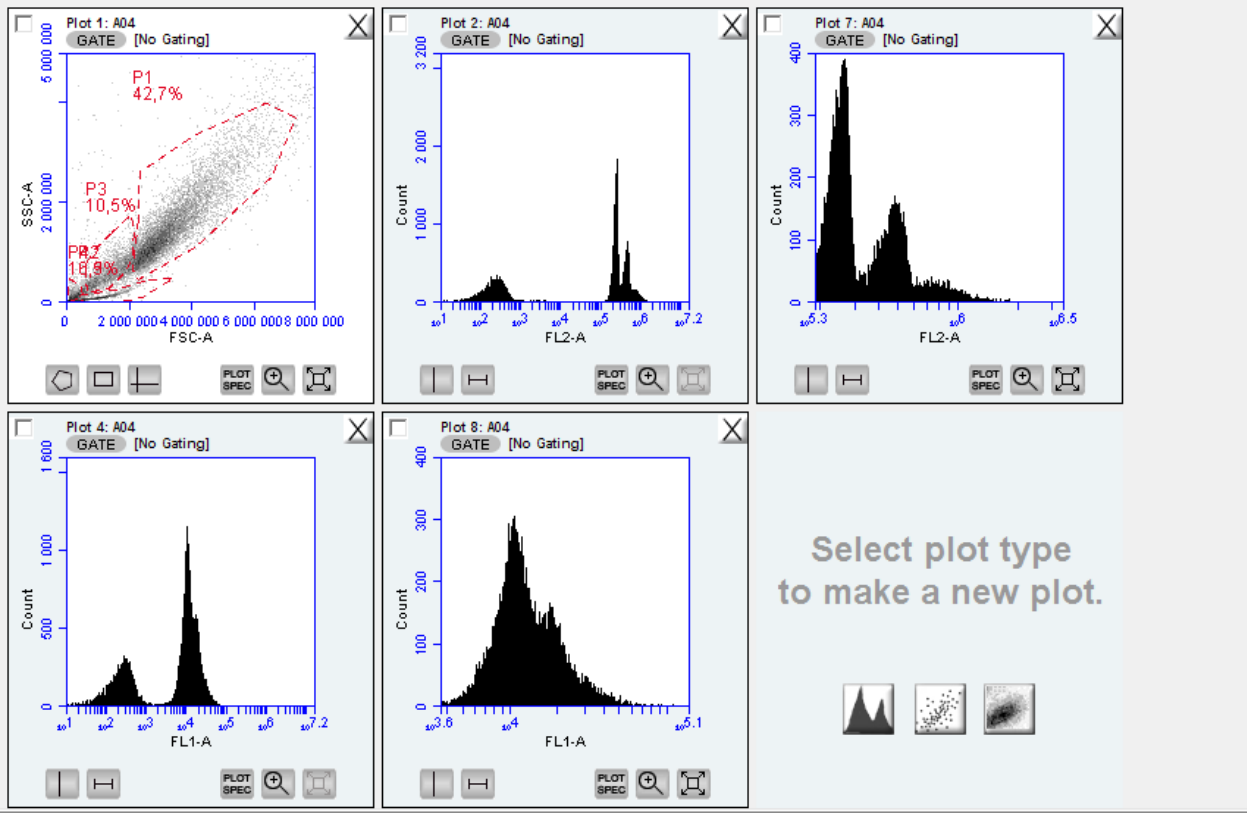
Plot 1: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	202	100,00%	100,00%	1 964 033,11	886 856,44	109,68%	150,89%		
P2	1 558	202	7,79%	7,79%	992 054,20	80 839,56	48,55%	82,45%		
P3	988	202	4,94%	4,94%	1 444 552,65	663 165,43	28,87%	40,60%		
P1	8 021	202	40,11%	40,11%	3 546 332,04	1 563 535,13	27,69%	41,82%		
P4	2 775	202	13,88%	13,88%	184 288,51	50 994,85	39,21%	131,09%		

Plot 2: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	202	100,00%	100,00%	225 057,71	160,30%	

Plot 4: A03	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	202	100,00%	100,00%	8 820,79	189,46%	

Figure 55. A-series sample Nr. 3. Stained with DAPI & PI. Prepared according to Appendix A28.

Appendix B29



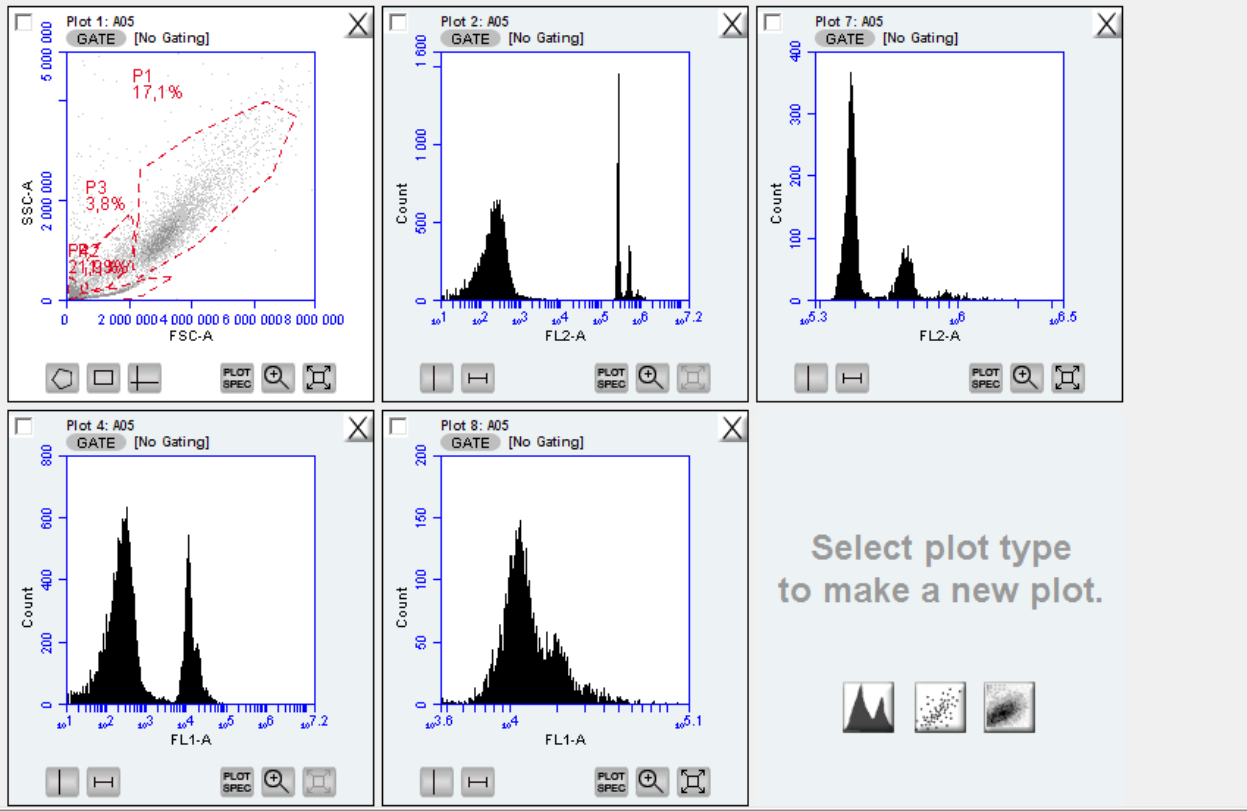
Plot 1: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	153	100,00%	100,00%	1 993 337,01	1 007 281,47	98,96%	182,07%		
P2	1 307	153	6,54%	6,54%	932 004,71	71 508,95	47,83%	79,70%		
P3	2 093	153	10,46%	10,46%	1 406 977,04	625 334,46	30,44%	39,81%		
P1	8 537	153	42,68%	42,68%	3 379 546,39	1 503 561,15	27,87%	41,75%		
P4	2 185	153	10,92%	10,92%	191 918,37	48 244,93	39,88%	125,31%		

Plot 2: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	153	100,00%	100,00%	222 630,12	120,38%	

Plot 4: A04	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	153	100,00%	100,00%	9 408,29	202,26%	

Figure 56. A-series sample Nr. 4. Stained with DAPI & PI. Prepared according to Appendix A29.

Appendix B30



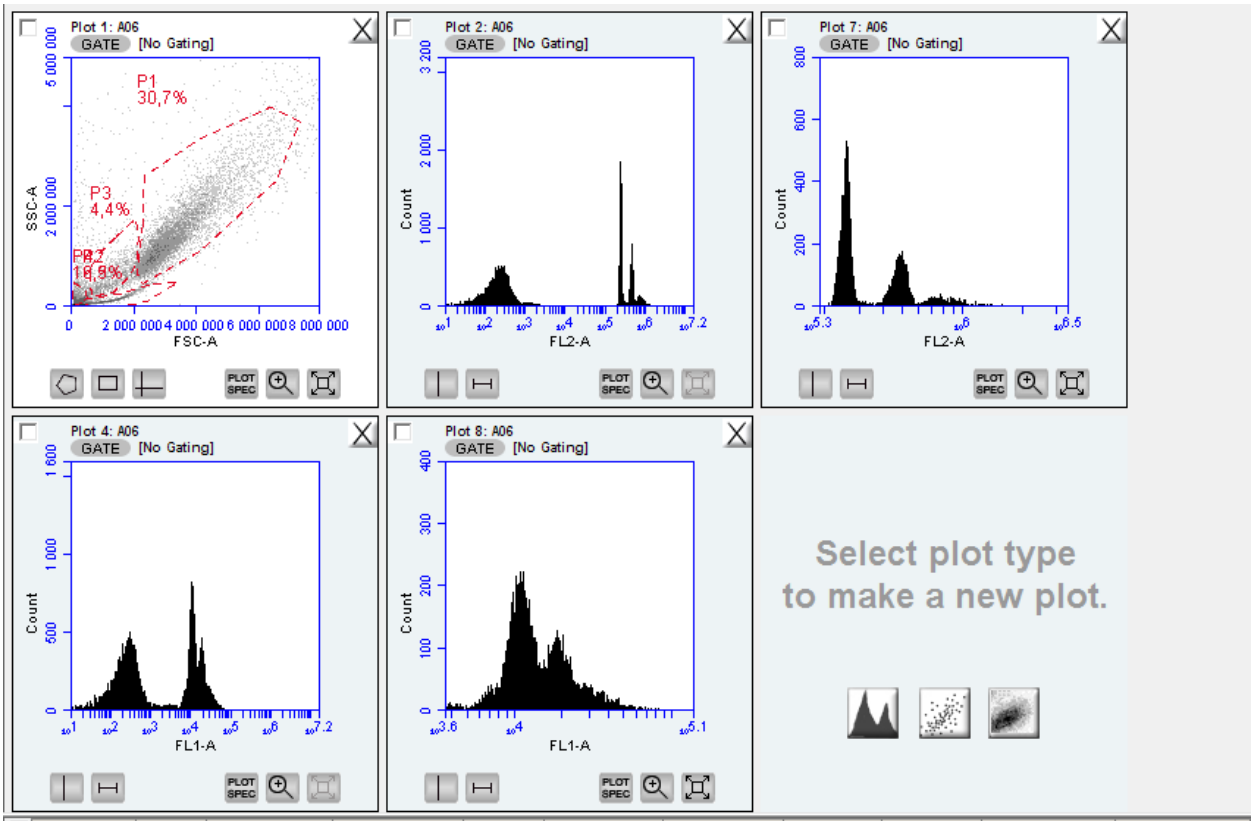
Plot 1: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	609	100,00%	100,00%	954 389,18	396 624,95	157,15%	238,25%		
P2	2 256	609	11,28%	11,28%	919 553,76	73 620,74	48,71%	81,62%		
P3	752	609	3,76%	3,76%	1 433 666,24	693 489,77	29,63%	39,46%		
P1	3 426	609	17,13%	17,13%	3 519 152,66	1 559 972,16	25,56%	39,97%		
P4	4 372	609	21,86%	21,86%	179 002,72	43 962,02	33,40%	145,35%		

Plot 2: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	609	100,00%	100,00%	85 969,36	237,51%	

Plot 4: A05	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	609	100,00%	100,00%	3 745,52	296,98%	

Figure 57. A-series sample Nr. 5. Stained with DAPI & PI. Prepared according to Appendix A30.

Appendix B31



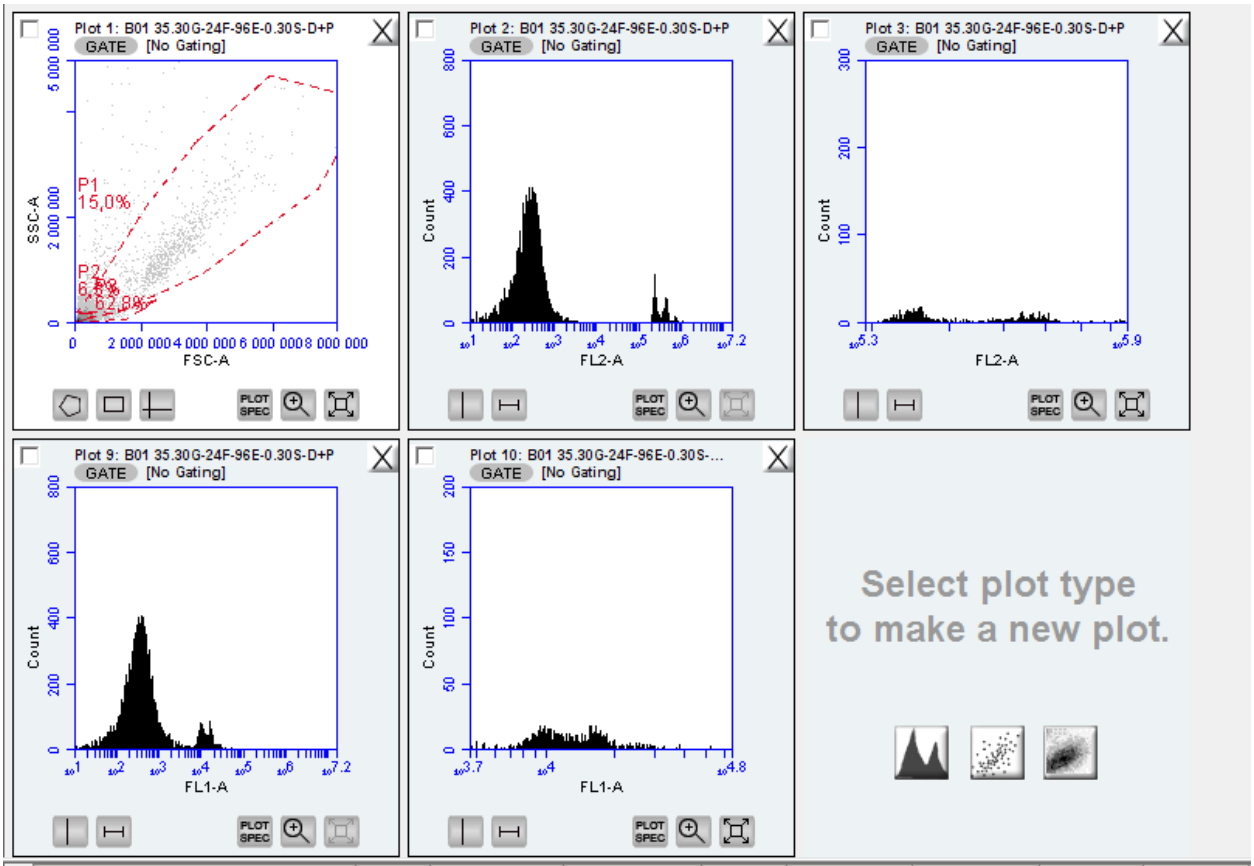
Plot 1: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A	Median FSC-A	Median SSC-A
All	20 000	224	100,00%	100,00%	1 478 330,48	647 428,21	122,60%	172,27%		
P2	1 979	224	9,90%	9,90%	956 250,42	74 394,10	47,31%	81,25%		
P3	873	224	4,36%	4,36%	1 344 160,72	604 484,27	30,92%	42,21%		
P1	6 147	224	30,74%	30,74%	3 425 806,25	1 491 377,56	28,65%	43,00%		
P4	3 300	224	16,50%	16,50%	186 648,86	49 362,75	39,34%	128,23%		

Plot 2: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	20 000	224	100,00%	100,00%	148 056,81	163,03%	

Plot 4: A06	Count	Volume (µL)	% of This Plot	% of All	Mean FL1-A	CV FL1-A	Median FL1-A
All	20 000	224	100,00%	100,00%	6 723,52	182,81%	

Figure 58. A-series sample Nr. 6. Stained with DAPI & PI. Prepared according to Appendix A31.

Appendix B32



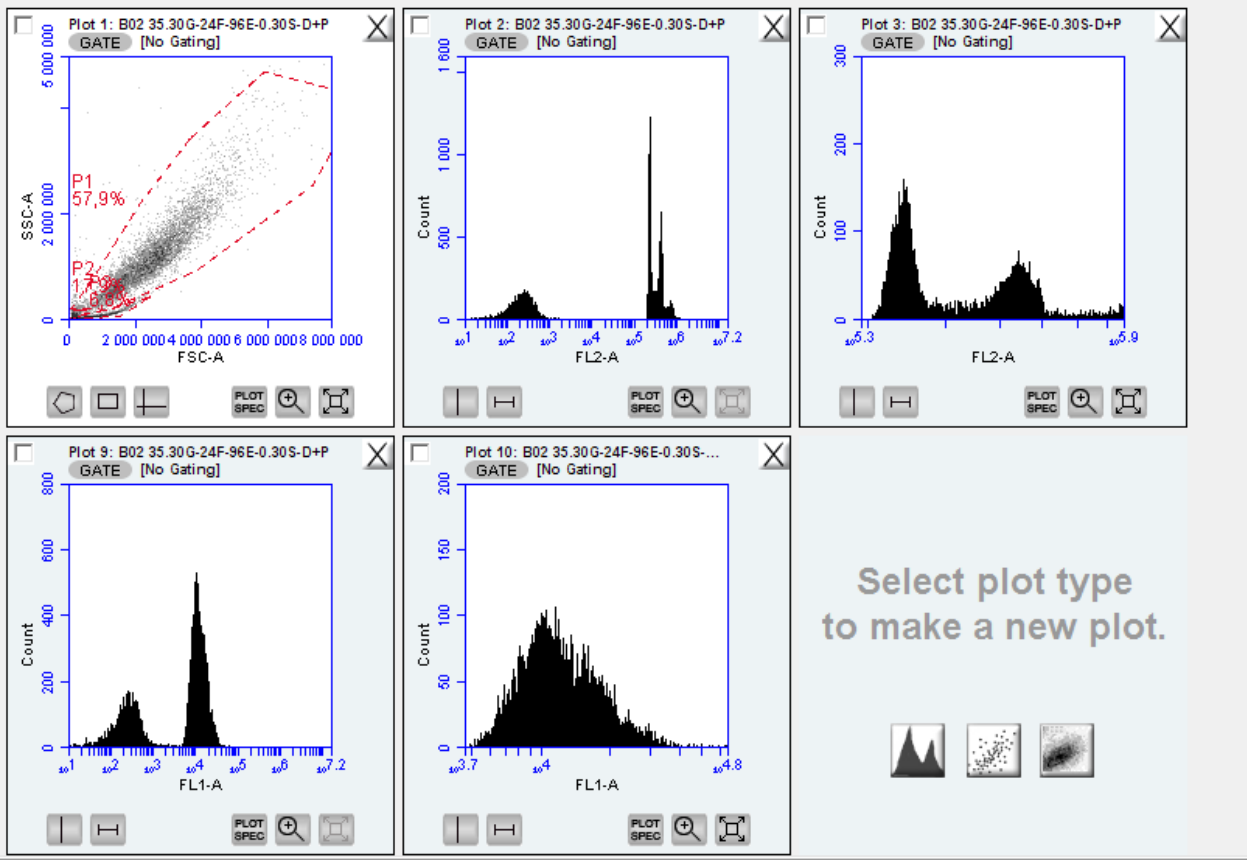
Plot 1: B01 35.30G-24F-96E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	13	100,00%	100,00%	471 084,65	242 539,51	200,65%	300,02%
P1	1 500	13	15,00%	15,00%	1 966 108,45	966 574,14	72,72%	78,77%
P2	630	13	6,30%	6,30%	462 845,27	43 608,30	62,86%	82,47%
P3	6 285	13	62,85%	62,85%	133 522,68	42 828,53	68,19%	84,89%

Plot 3: B01 35.30G-24F-96E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	13	100,00%	100,00%	29 720,58	387,39%	

Plot 2: B01 35.30G-24F-96E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	13	100,00%	100,00%	29 720,58	387,39%	

Figure 59. B-series sample Nr. 1. Stained with DAPI & PI. Prepared according to Appendix A32.

Appendix B33



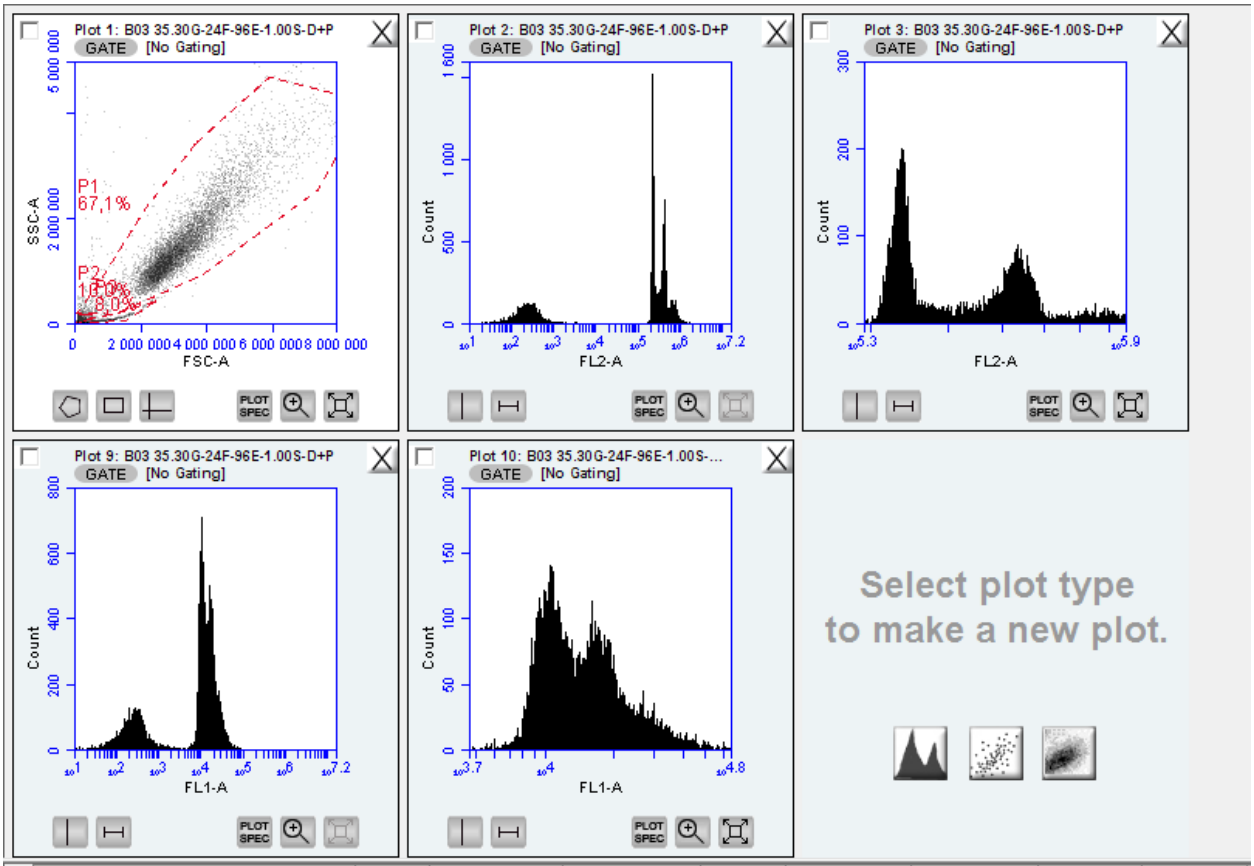
Plot 1: B02 35.30G-24F-96E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	70	100,00%	100,00%	1 659 380,37	780 570,48	91,24%	132,26%
P1	5 786	70	57,86%	57,86%	2 555 215,75	1 230 164,86	42,34%	54,07%
P2	1 793	70	17,93%	17,93%	566 828,38	43 285,75	78,03%	116,77%
P3	683	70	6,83%	6,83%	121 485,17	44 504,31	81,10%	96,32%

Plot 3: B02 35.30G-24F-96E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	70	100,00%	100,00%	213 394,78	114,40%	

Plot 2: B02 35.30G-24F-96E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	70	100,00%	100,00%	213 394,78	114,40%	

Figure 60. B-series sample Nr. 2. Stained with DAPI & PI. Prepared according to Appendix A32.

Appendix B34



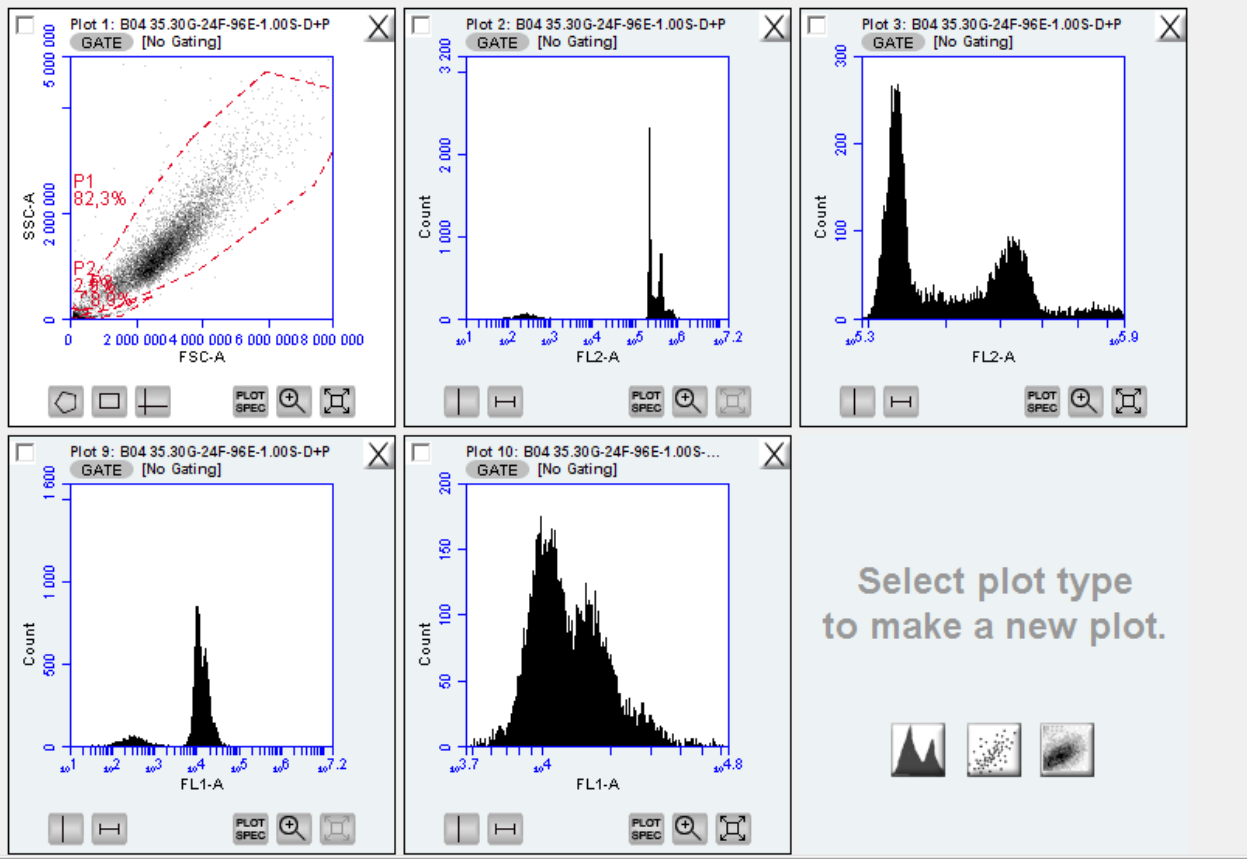
Plot 1: B03 35.30G-24F-96E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	103	100,00%	100,00%	2 430 946,49	1 161 624,68	78,19%	99,65%
P1	6 709	103	67,09%	67,09%	3 281 477,10	1 542 587,58	35,56%	48,06%
P2	998	103	9,98%	9,98%	555 710,02	40 766,03	80,44%	126,59%
P3	800	103	8,00%	8,00%	124 527,57	51 296,76	73,09%	82,70%

Plot 3: B03 35.30G-24F-96E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	103	100,00%	100,00%	262 203,77	99,39%	

Plot 2: B03 35.30G-24F-96E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	103	100,00%	100,00%	262 203,77	99,39%	

Figure 61. B-series sample Nr. 3. Stained with DAPI & PI. Prepared according to Appendix A33.

Appendix B35



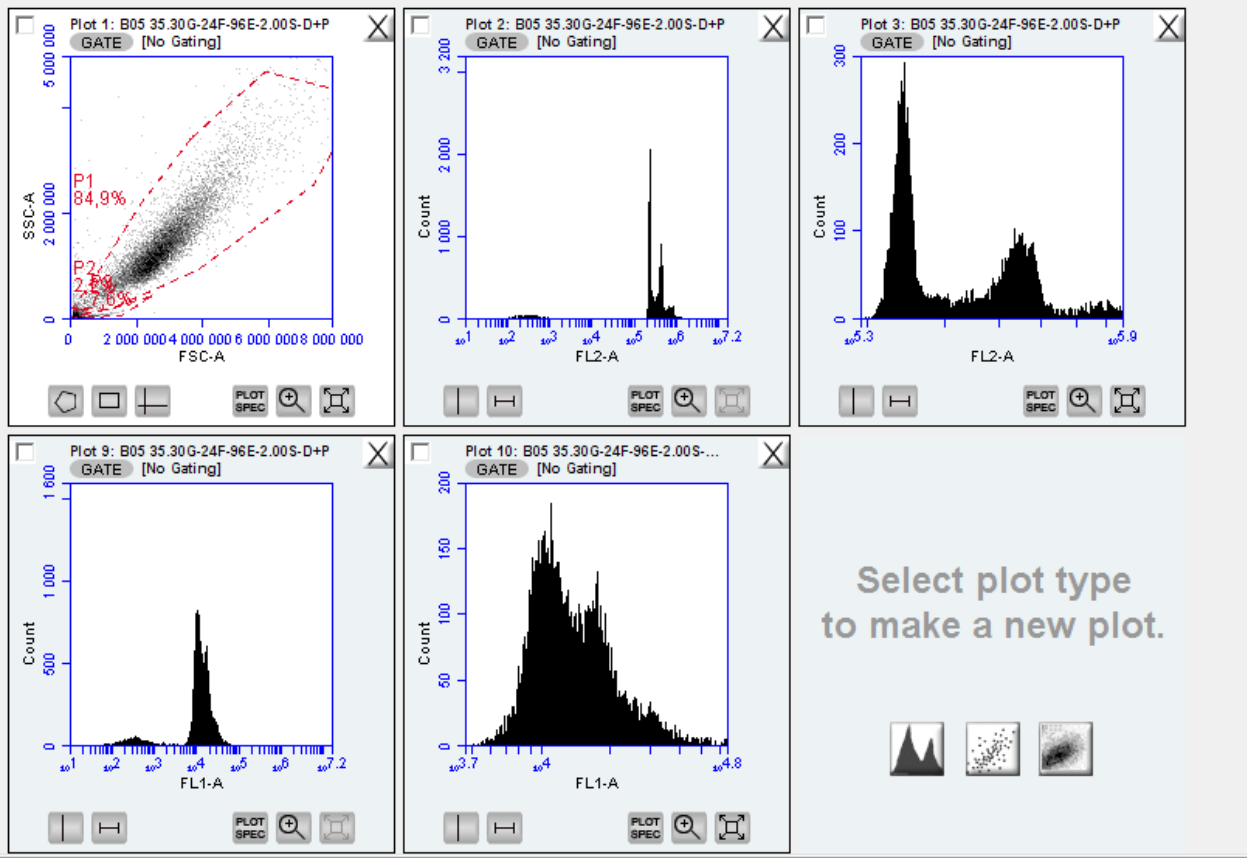
Plot 1: B04 35.30G-24F-96E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	119	100,00%	100,00%	2 431 264,60	1 183 258,21	59,90%	75,81%
P1	8 232	119	82,32%	82,32%	2 837 225,71	1 364 902,30	36,15%	46,74%
P2	291	119	2,91%	2,91%	541 269,39	40 700,44	80,22%	117,83%
P3	888	119	8,88%	8,88%	123 025,97	46 398,16	73,65%	82,34%

Plot 3: B04 35.30G-24F-96E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	119	100,00%	100,00%	288 494,44	75,34%	

Plot 2: B04 35.30G-24F-96E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	119	100,00%	100,00%	288 494,44	75,34%	

Figure 62. B-series sample Nr. 4. Stained with DAPI & PI. Prepared according to Appendix A33.

Appendix B36



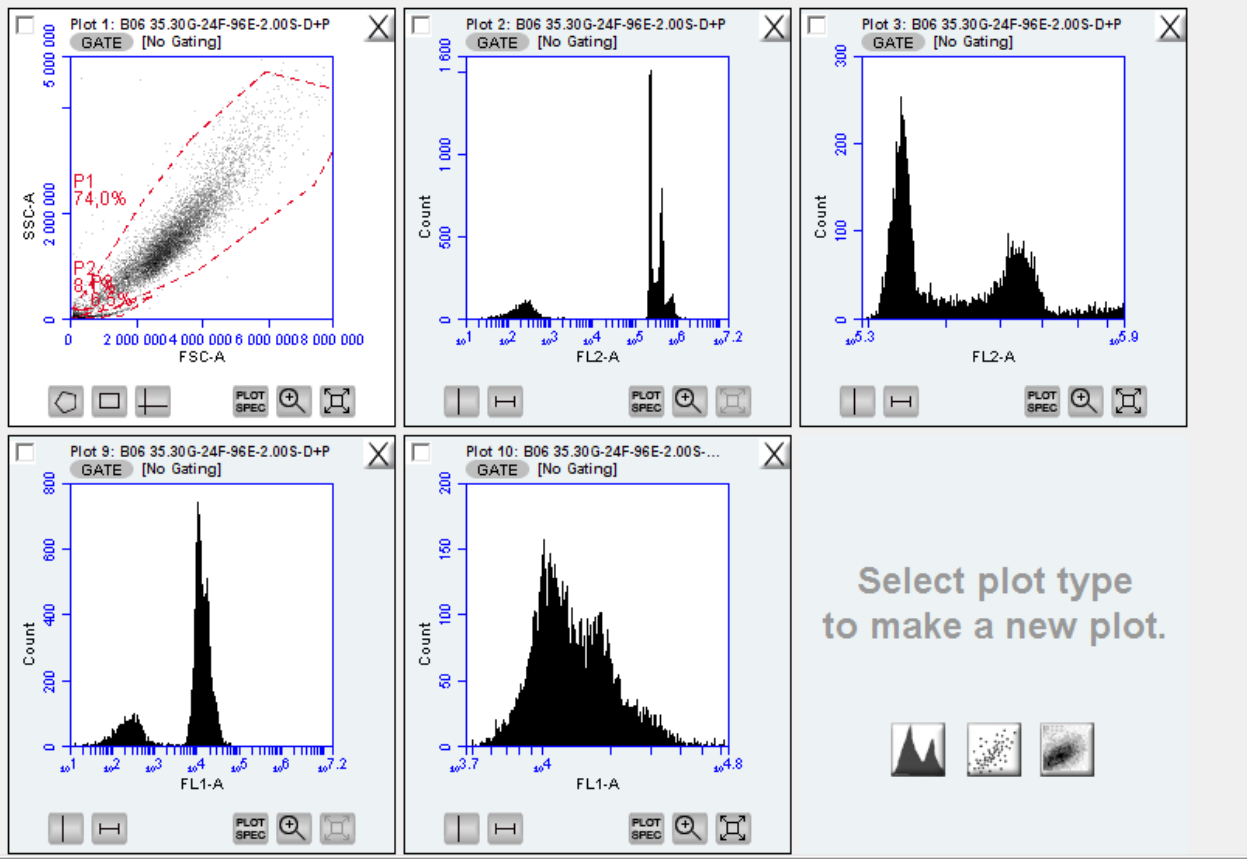
Plot 1: B05 35.30G-24F-96E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	82	100,00%	100,00%	2 565 849,59	1 338 781,83	61,59%	81,04%
P1	8 491	82	84,91%	84,91%	2 834 682,92	1 436 013,45	38,53%	48,36%
P2	219	82	2,19%	2,19%	499 942,48	34 336,75	69,61%	89,98%
P3	765	82	7,65%	7,65%	124 605,40	51 532,67	70,04%	77,20%

Plot 3: B05 35.30G-24F-96E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	82	100,00%	100,00%	331 951,18	81,50%	

Plot 2: B05 35.30G-24F-96E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	82	100,00%	100,00%	331 951,18	81,50%	

Figure 63. B-series sample Nr. 5. Stained with DAPI & PI. Prepared according to Appendix A34.

Appendix B37



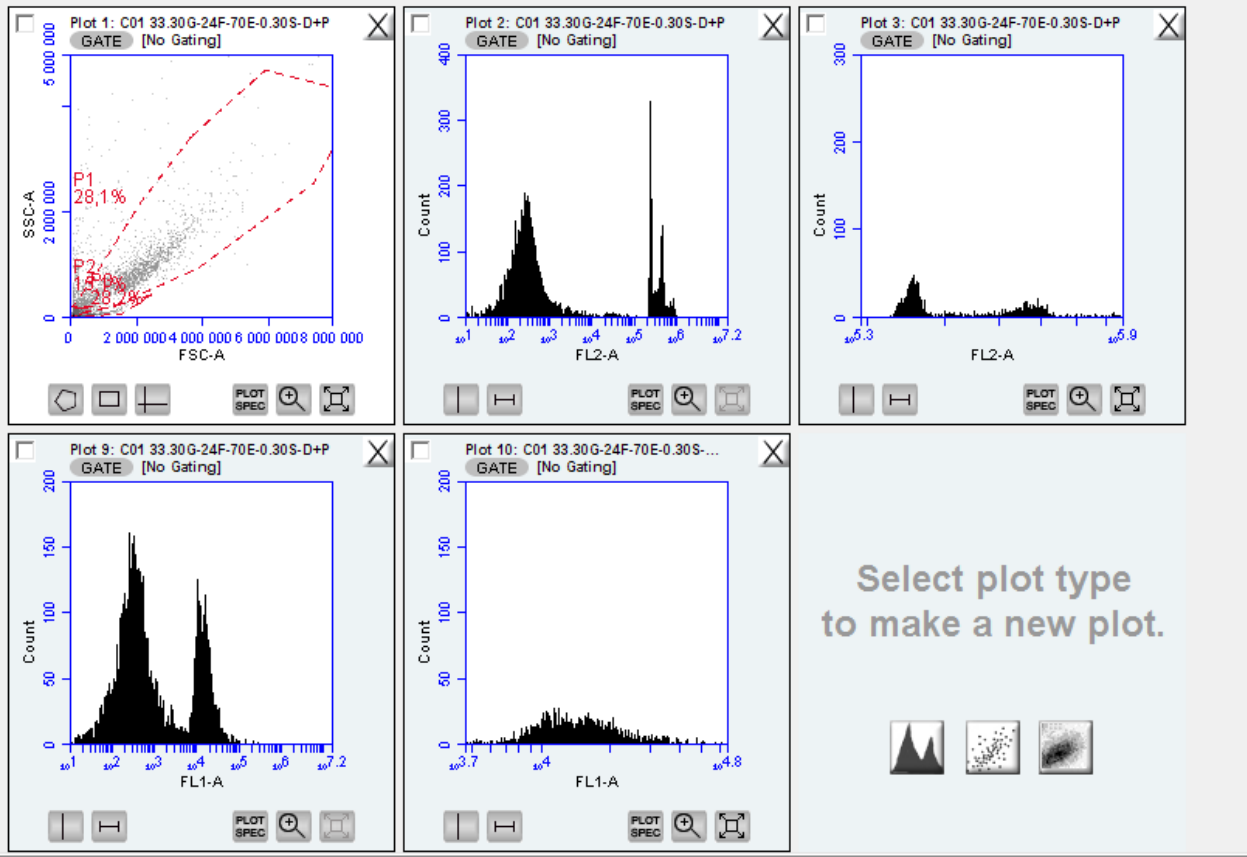
Plot 1: B06 35.30G-24F-96E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	121	100,00%	100,00%	2 393 139,36	1 199 038,02	69,20%	86,61%
P1	7 403	121	74,03%	74,03%	3 048 228,87	1 525 563,12	36,67%	47,75%
P2	814	121	8,14%	8,14%	516 035,71	36 158,94	76,03%	109,43%
P3	652	121	6,52%	6,52%	122 392,97	45 306,10	75,10%	89,32%

Plot 3: B06 35.30G-24F-96E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	121	100,00%	100,00%	277 352,98	85,82%	

Plot 2: B06 35.30G-24F-96E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	121	100,00%	100,00%	277 352,98	85,82%	

Figure 64. B-series sample Nr. 6. Stained with DAPI & PI. Prepared according to Appendix A34.

Appendix B38



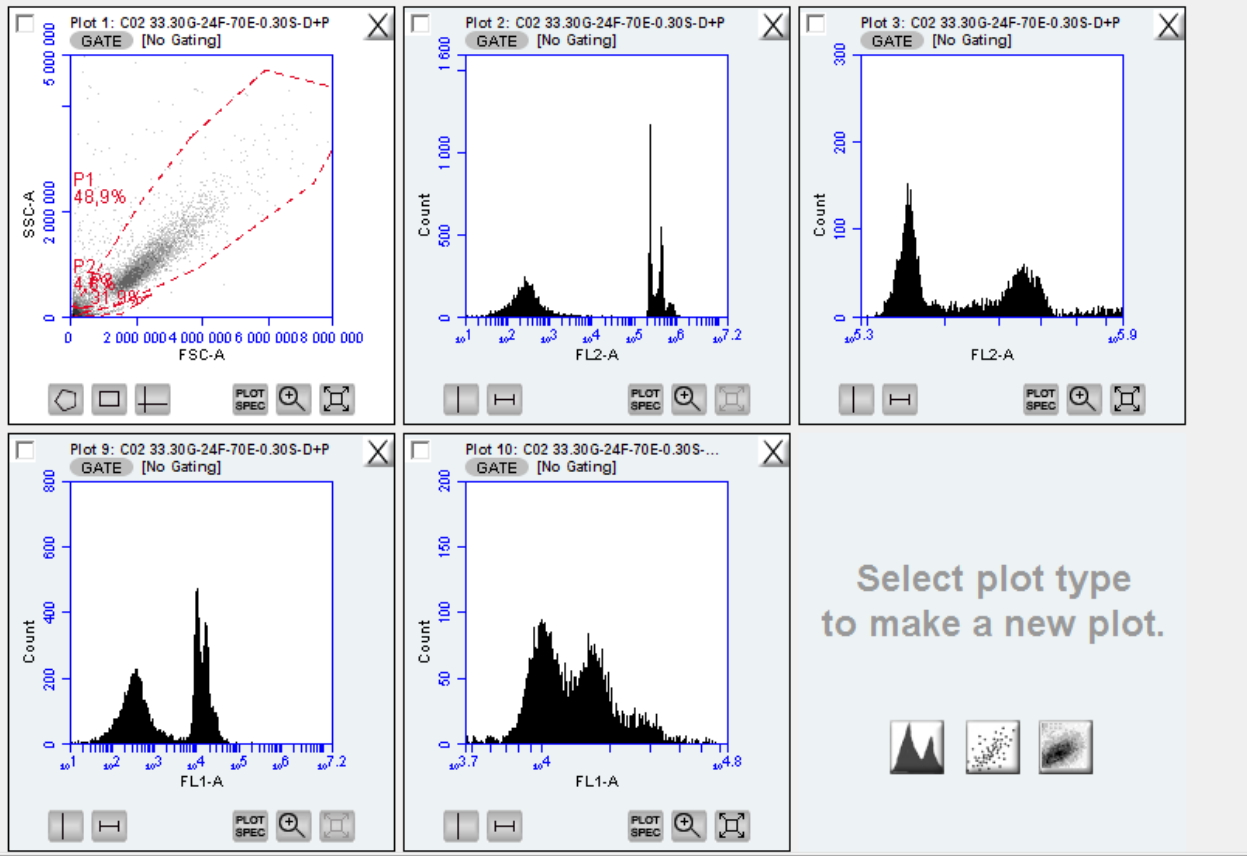
Plot 1: C01 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	5 699	212	100,00%	100,00%	746 063,18	403 757,54	151,35%	248,52%
P1	1 601	212	28,09%	28,09%	1 956 928,27	897 035,69	55,27%	59,32%
P2	859	212	15,07%	15,07%	446 296,59	30 890,69	70,44%	100,86%
P3	1 607	212	28,20%	28,20%	128 977,87	51 416,84	71,82%	80,99%

Plot 3: C01 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	5 699	212	100,00%	100,00%	89 174,50	212,77%	

Plot 2: C01 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	5 699	212	100,00%	100,00%	89 174,50	212,77%	

Figure 65. C-series sample Nr. 1. Stained with DAPI & PI. Prepared according to Appendix A35.

Appendix B39



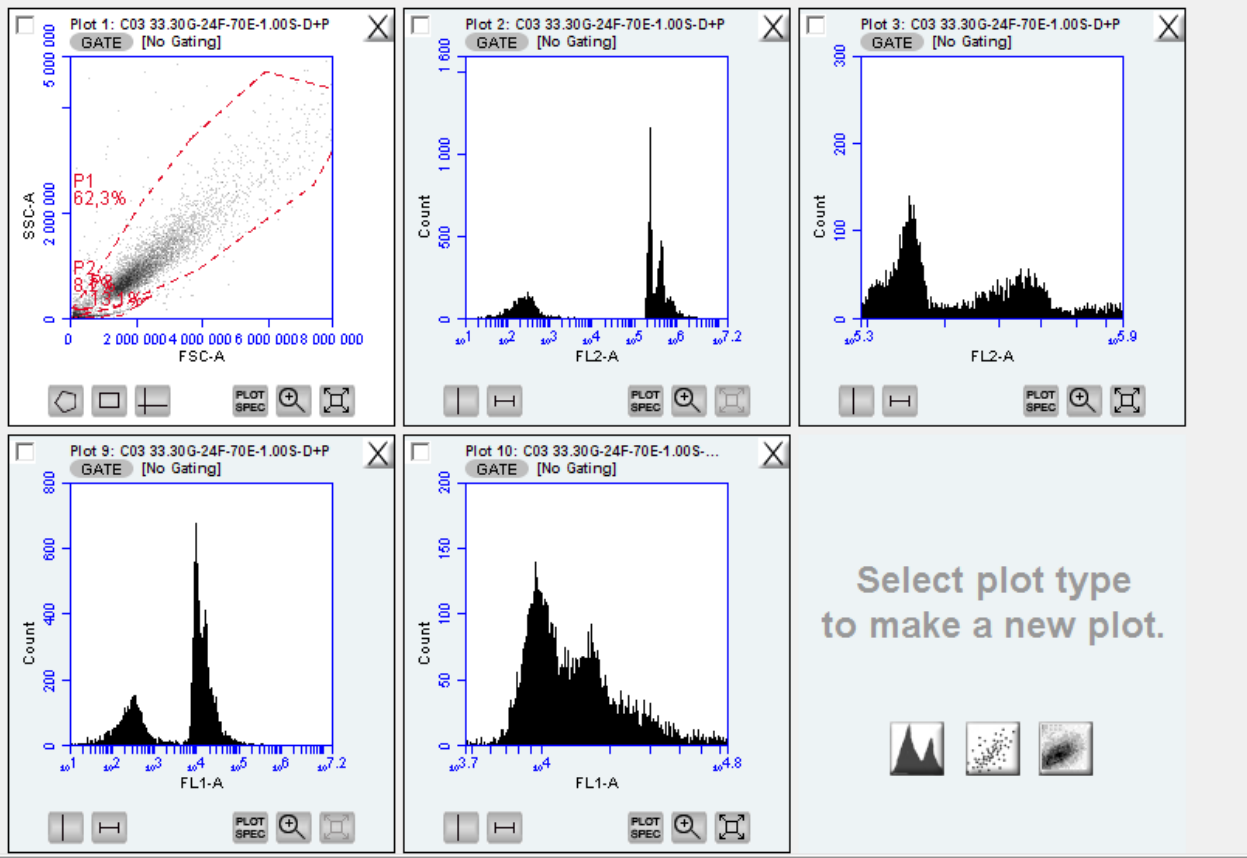
Plot 1: C02 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 009	162	100,00%	100,00%	1 241 454,80	652 184,93	122,38%	200,23%
P1	4 893	162	48,89%	48,89%	2 179 019,42	981 096,89	44,16%	51,46%
P2	481	162	4,81%	4,81%	443 784,78	33 629,98	76,18%	109,76%
P3	3 195	162	31,92%	31,92%	118 087,86	48 418,17	66,96%	83,35%

Plot 3: C02 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 009	162	100,00%	100,00%	183 853,88	183,33%	

Plot 2: C02 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 009	162	100,00%	100,00%	183 853,88	183,33%	

Figure 66. C-series sample Nr. 2. Stained with DAPI & PI. Prepared according to Appendix A35.

Appendix B40



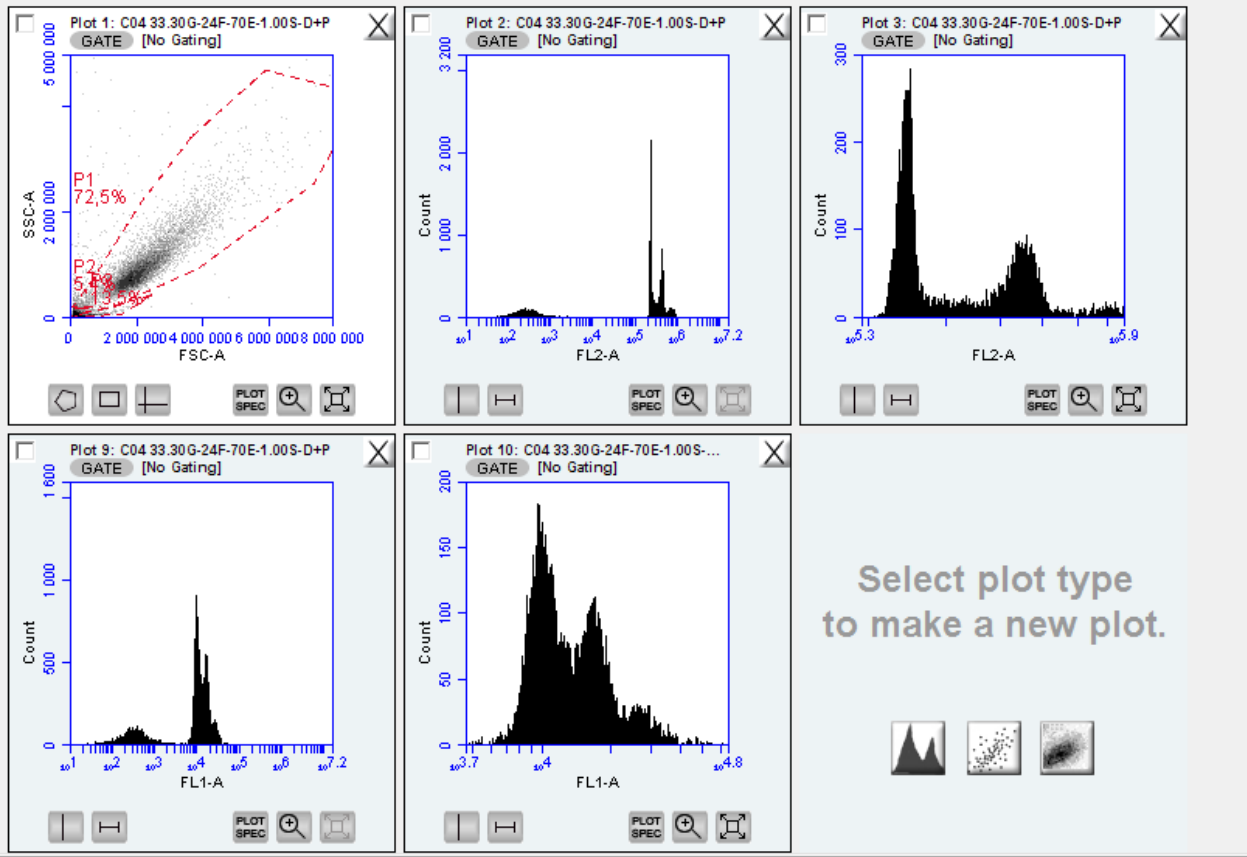
Plot 1: C03 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	393	100,00%	100,00%	1 748 273,72	847 021,40	119,77%	181,53%
P1	6 229	393	62,29%	62,29%	2 258 513,23	1 006 522,10	51,27%	60,34%
P2	824	393	8,24%	8,24%	487 024,85	33 577,38	80,81%	120,59%
P3	1 306	393	13,06%	13,06%	121 631,11	44 059,11	71,92%	88,20%

Plot 3: C03 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	393	100,00%	100,00%	308 044,38	195,66%	

Plot 2: C03 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	393	100,00%	100,00%	308 044,38	195,66%	

Figure 67. C-series sample Nr. 3. Stained with DAPI & PI. Prepared according to Appendix A36.

Appendix B41



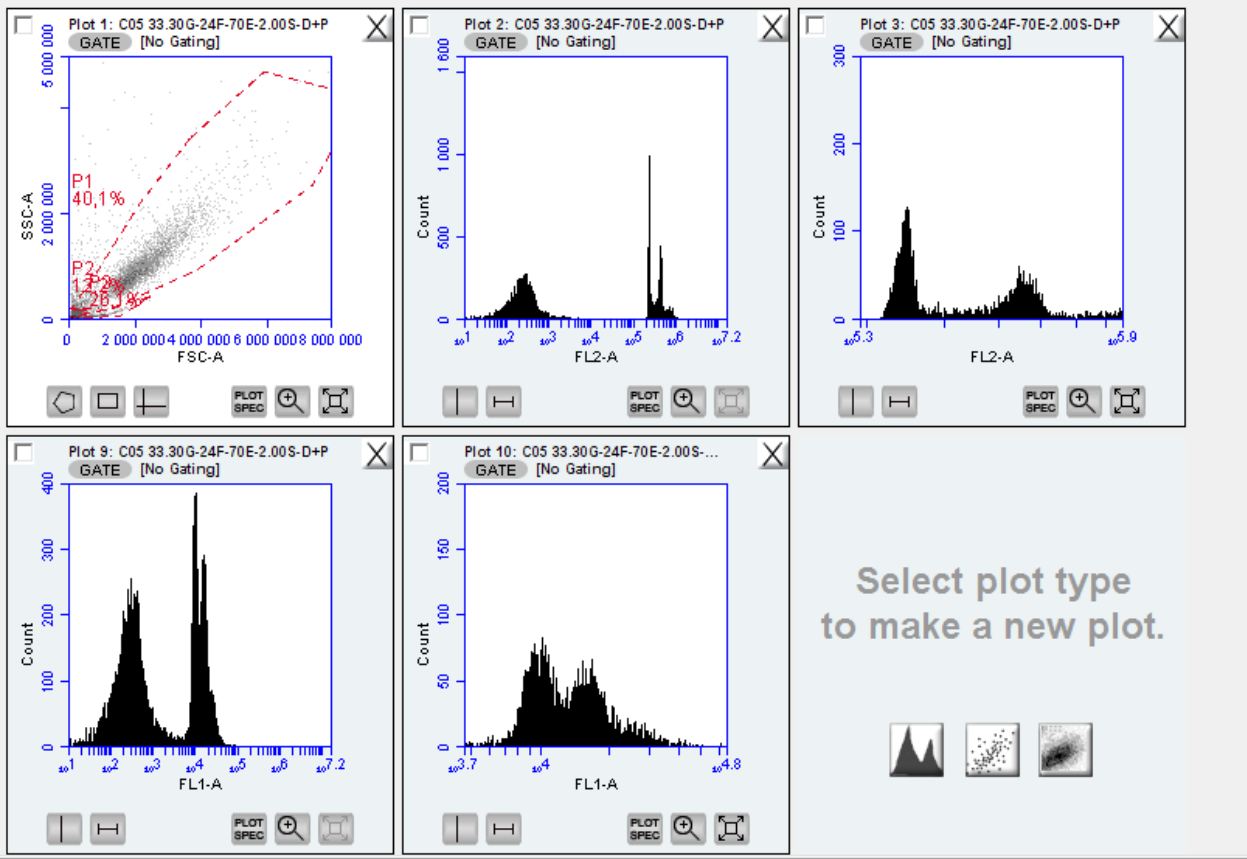
Plot 1: C04 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	195	100,00%	100,00%	1 694 488,68	775 378,86	74,53%	106,83%
P1	7 252	195	72,52%	72,52%	2 205 498,98	977 977,09	40,93%	50,21%
P2	538	195	5,38%	5,38%	520 028,96	38 422,95	73,61%	111,33%
P3	1 353	195	13,53%	13,53%	126 665,64	50 943,37	67,14%	78,66%

Plot 3: C04 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	195	100,00%	100,00%	263 926,47	89,11%	

Plot 2: C04 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	195	100,00%	100,00%	263 926,47	89,11%	

Figure 68. C-series sample Nr. 4. Stained with DAPI & PI. Prepared according to Appendix A36.

Appendix B42



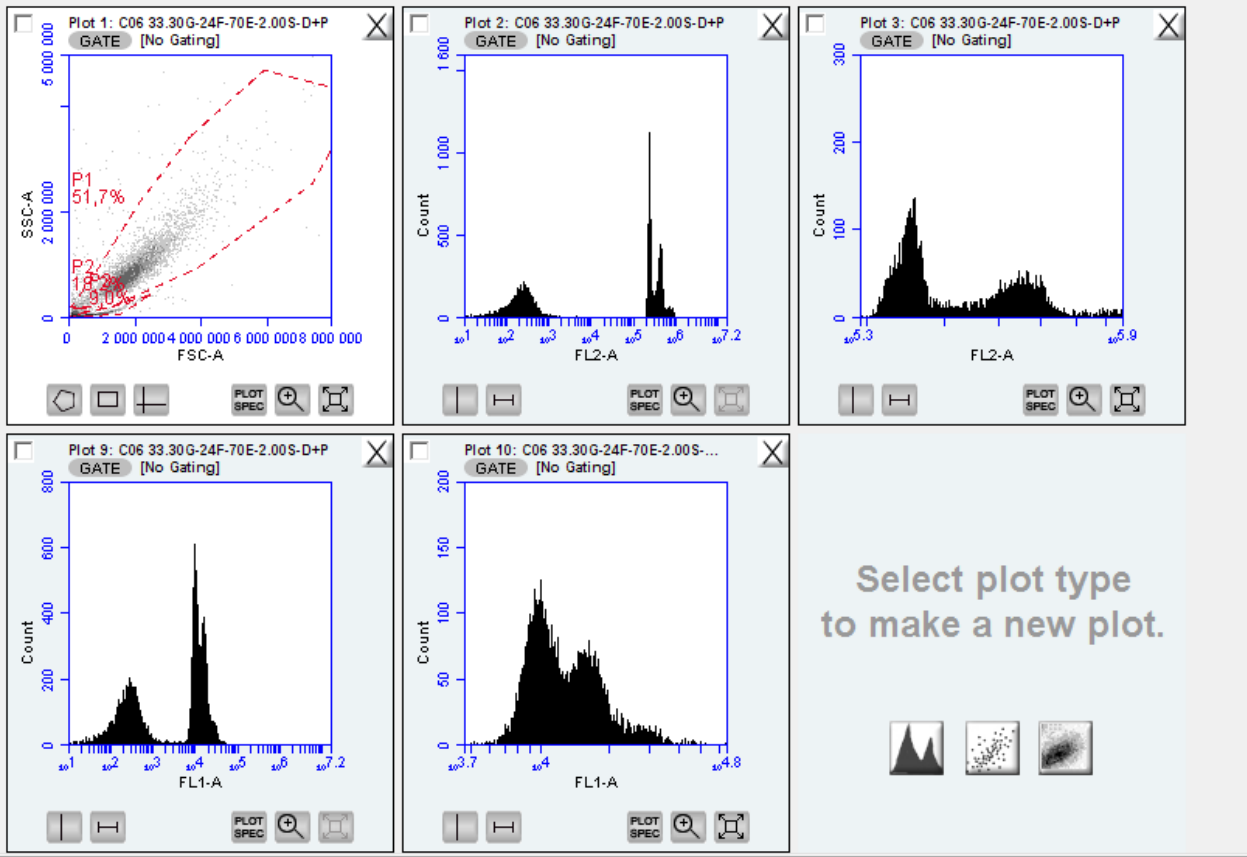
Plot 1: C05 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	129	100,00%	100,00%	1 023 441,05	496 897,42	120,02%	175,59%
P1	4 012	129	40,12%	40,12%	2 177 296,83	1 007 577,13	44,77%	52,79%
P2	1 218	129	12,18%	12,18%	451 083,18	32 185,35	75,36%	108,88%
P3	2 611	129	26,11%	26,11%	123 266,16	46 355,91	71,10%	85,12%

Plot 3: C05 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	129	100,00%	100,00%	143 979,94	163,10%	

Plot 2: C05 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	129	100,00%	100,00%	143 979,94	163,10%	

Figure 69. C-series sample Nr. 5. Stained with DAPI & PI. Prepared according to Appendix A37.

Appendix B43



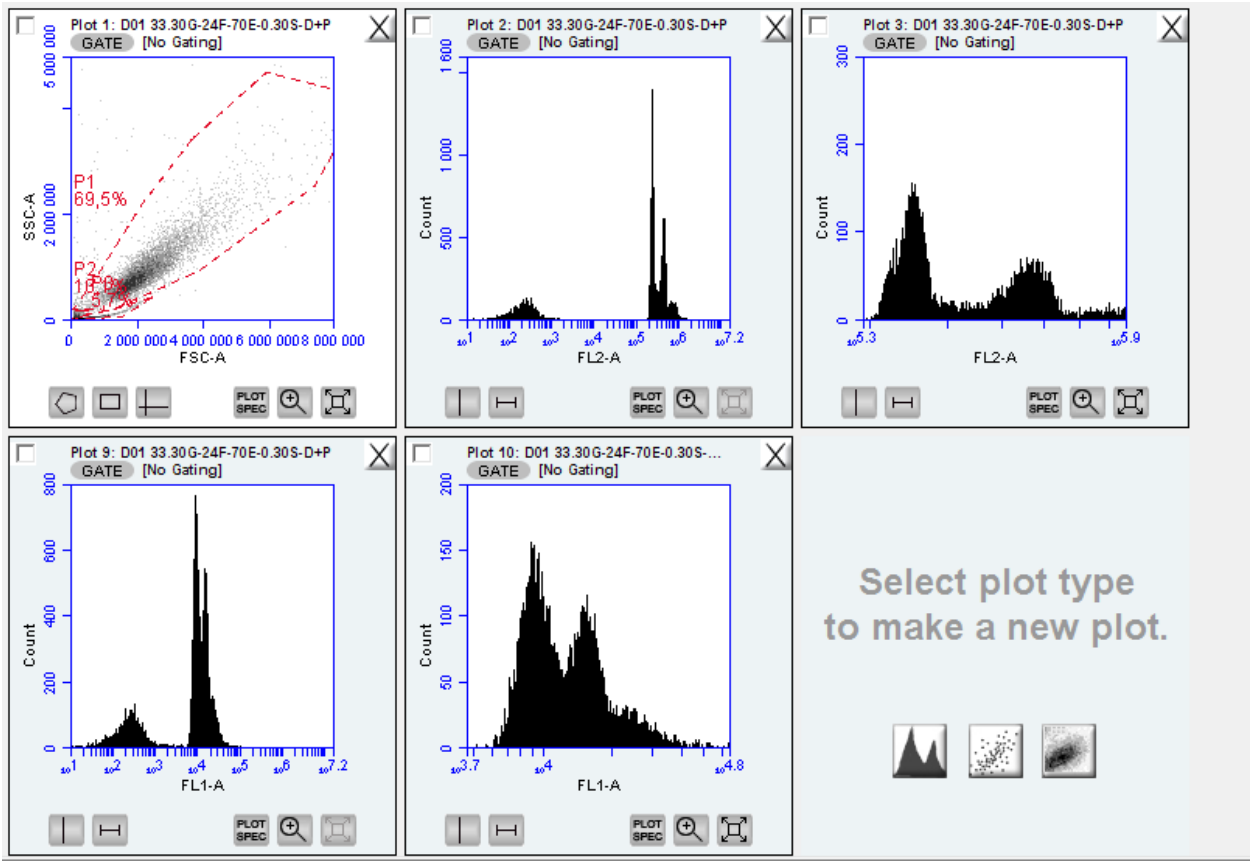
Plot 1: C06 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	199	100,00%	100,00%	1 185 919,65	554 683,28	95,21%	154,59%
P1	5 170	199	51,70%	51,70%	1 994 068,56	937 869,74	40,34%	48,96%
P2	1 822	199	18,22%	18,22%	468 228,61	32 331,80	74,72%	111,09%
P3	903	199	9,03%	9,03%	124 282,83	43 166,89	73,26%	88,21%

Plot 3: C06 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	199	100,00%	100,00%	184 948,47	120,58%	

Plot 2: C06 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	199	100,00%	100,00%	184 948,47	120,58%	

Figure 70. C-series sample Nr. 6. Stained with DAPI & PI. Prepared according to Appendix A37.

Appendix B44



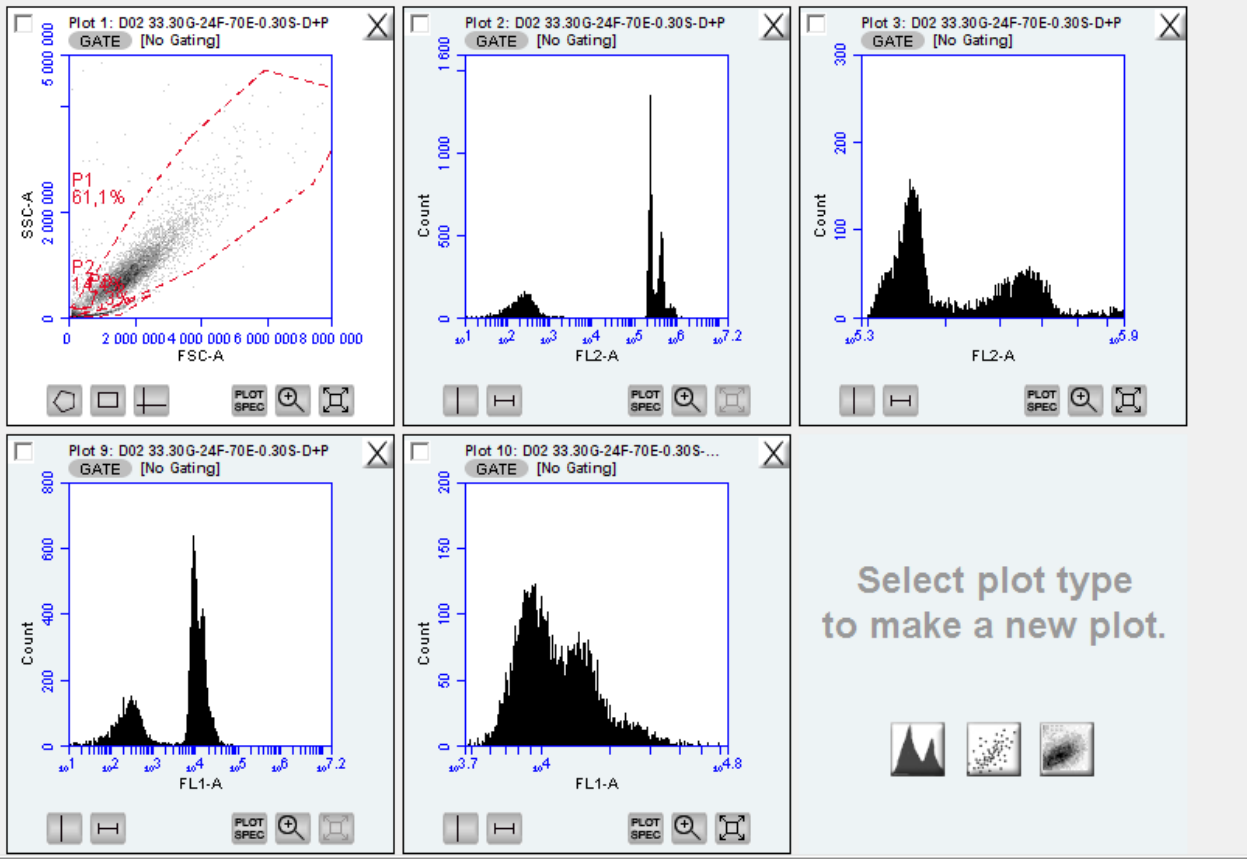
Plot 1: D01 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	244	100,00%	100,00%	1 757 960,12	752 727,48	82,58%	126,18%
P1	6 953	244	69,53%	69,53%	2 295 919,25	956 993,25	41,29%	50,21%
P2	1 013	244	10,13%	10,13%	531 182,85	39 805,70	83,85%	137,87%
P3	566	244	5,66%	5,66%	128 590,72	48 044,24	69,36%	87,92%

Plot 3: D01 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	244	100,00%	100,00%	281 057,88	142,42%	

Plot 2: D01 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	244	100,00%	100,00%	281 057,88	142,42%	

Figure 71. D-series sample Nr. 1. Stained with DAPI & PI. Prepared according to Appendix A37.

Appendix B45



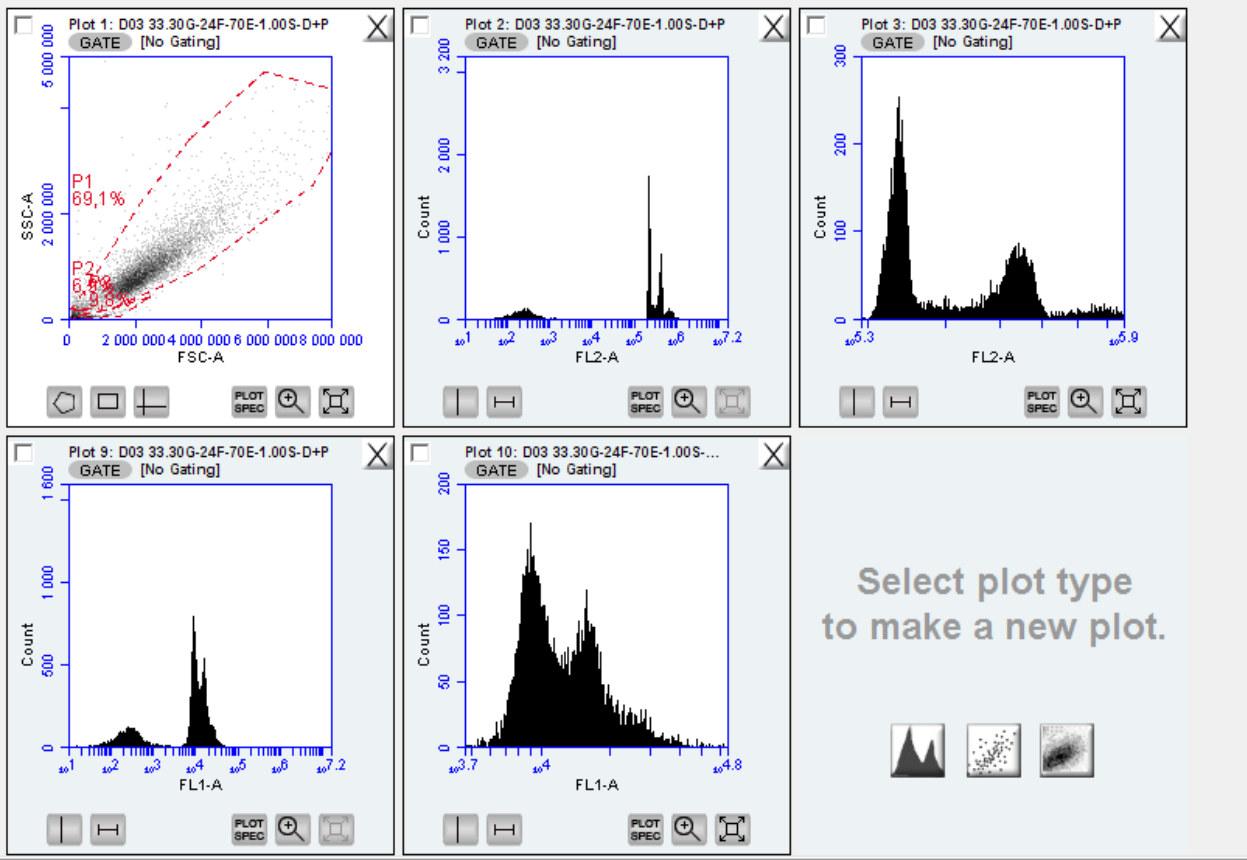
Plot 1: D02 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	231	100,00%	100,00%	1 325 329,51	620 419,31	90,35%	135,67%
P1	6 113	231	61,13%	61,13%	1 924 083,33	910 077,13	44,53%	52,61%
P2	1 435	231	14,35%	14,35%	538 567,87	42 084,71	72,70%	108,52%
P3	729	231	7,29%	7,29%	115 979,41	40 071,98	74,11%	102,21%

Plot 3: D02 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	231	100,00%	100,00%	222 080,84	110,84%	

Plot 2: D02 33.30G-24F-70E-0.30S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	231	100,00%	100,00%	222 080,84	110,84%	

Figure 72. D-series sample Nr. 2. Stained with DAPI & PI. Prepared according to Appendix A37.

Appendix B46



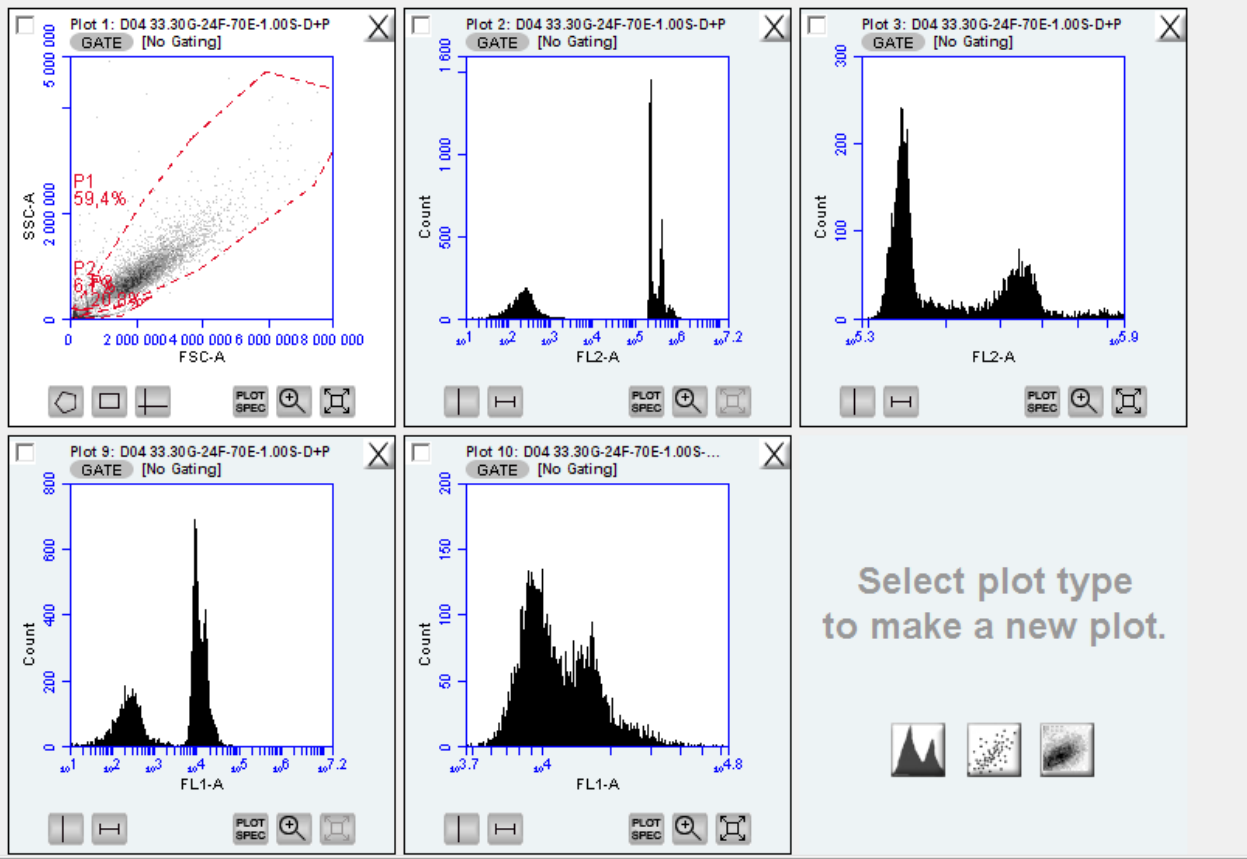
Plot 1: D03 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	207	100,00%	100,00%	1 773 972,18	737 874,43	80,63%	112,53%
P1	6 913	207	69,13%	69,13%	2 406 917,38	983 088,02	42,90%	52,19%
P2	688	207	6,88%	6,88%	432 910,05	29 616,74	77,76%	118,94%
P3	981	207	9,81%	9,81%	137 908,00	47 960,99	72,28%	85,80%

Plot 3: D03 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	207	100,00%	100,00%	249 533,86	96,80%	

Plot 2: D03 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	207	100,00%	100,00%	249 533,86	96,80%	

Figure 73. D-series sample Nr. 3. Stained with DAPI & PI. Prepared according to Appendix A38.

Appendix B47



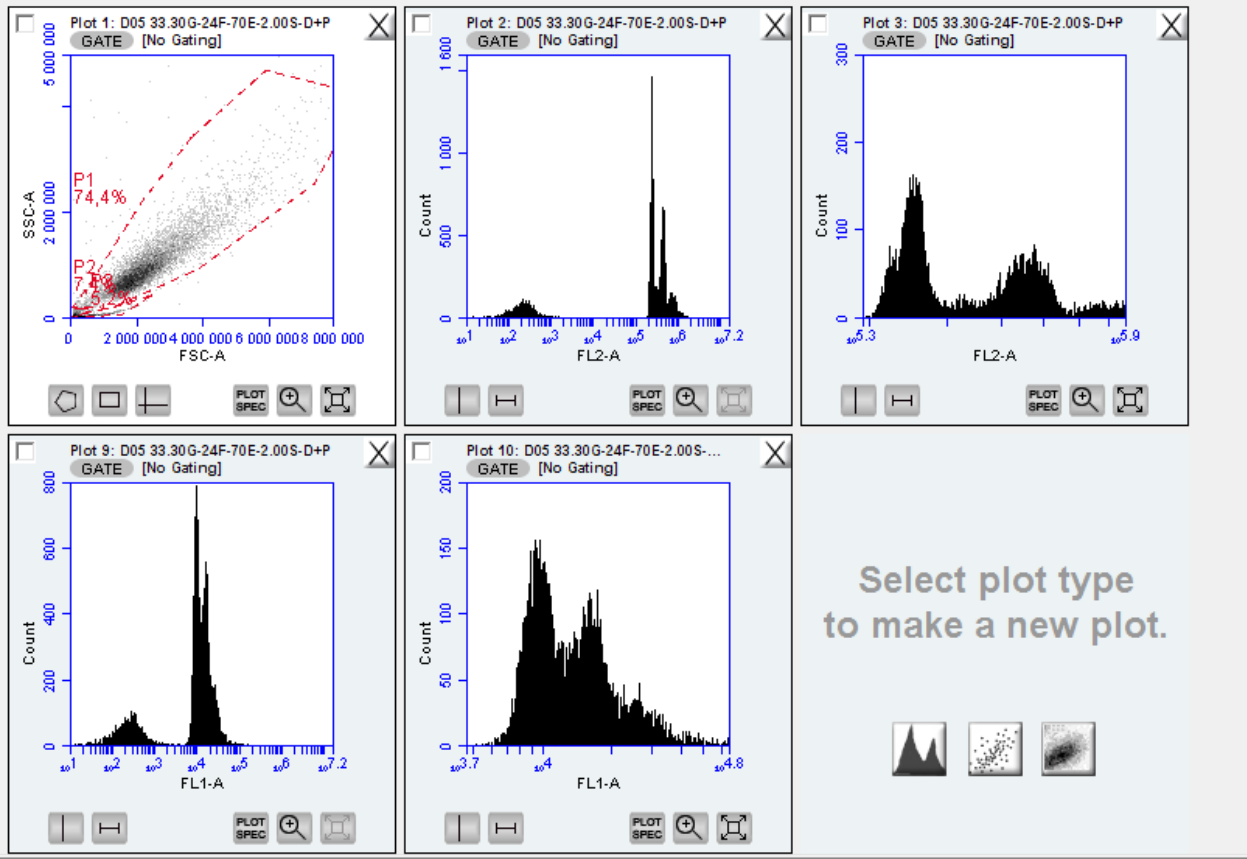
Plot 1: D04 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	187	100,00%	100,00%	1 384 436,04	564 370,30	92,61%	121,14%
P1	5 943	187	59,43%	59,43%	2 133 669,96	865 296,84	43,74%	49,74%
P2	671	187	6,71%	6,71%	456 678,54	31 755,64	80,59%	130,74%
P3	2 076	187	20,76%	20,76%	173 448,01	44 319,85	74,26%	84,10%

Plot 3: D04 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	187	100,00%	100,00%	206 625,28	116,00%	

Plot 2: D04 33.30G-24F-70E-1.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	187	100,00%	100,00%	206 625,28	116,00%	

Figure 74. D-series sample Nr. 4. Stained with DAPI & PI. Prepared according to Appendix A38.

Appendix B48



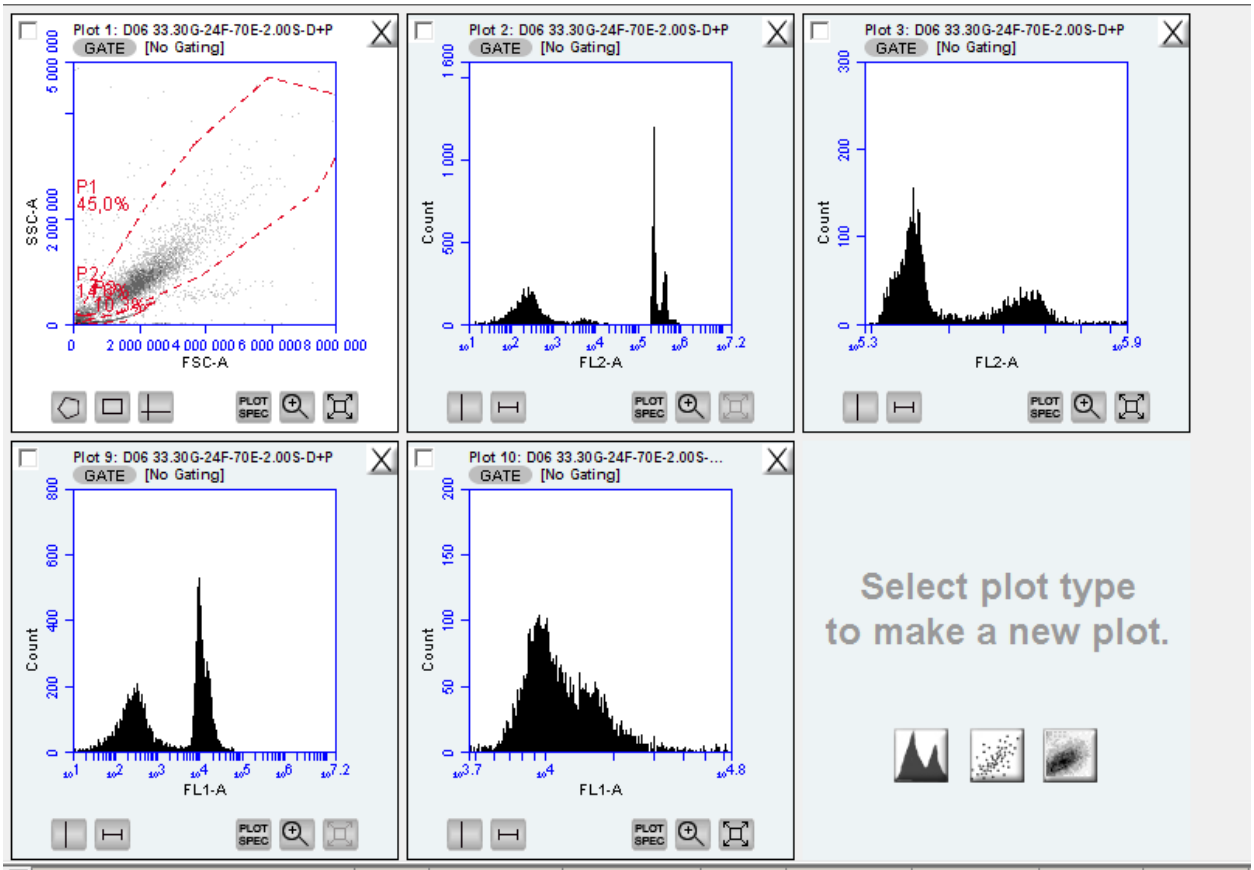
Plot 1: D05 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	222	100,00%	100,00%	1 951 952,22	852 785,36	85,47%	129,72%
P1	7 442	222	74,42%	74,42%	2 371 430,83	997 648,29	45,11%	52,99%
P2	739	222	7,39%	7,39%	478 271,57	30 698,51	78,52%	107,84%
P3	520	222	5,20%	5,20%	130 543,32	46 111,61	71,29%	90,57%

Plot 3: D05 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	222	100,00%	100,00%	318 487,36	107,84%	

Plot 2: D05 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	222	100,00%	100,00%	318 487,36	107,84%	

Figure 75. D-series sample Nr. 5. Stained with DAPI & PI. Prepared according to Appendix A39.

Appendix B49



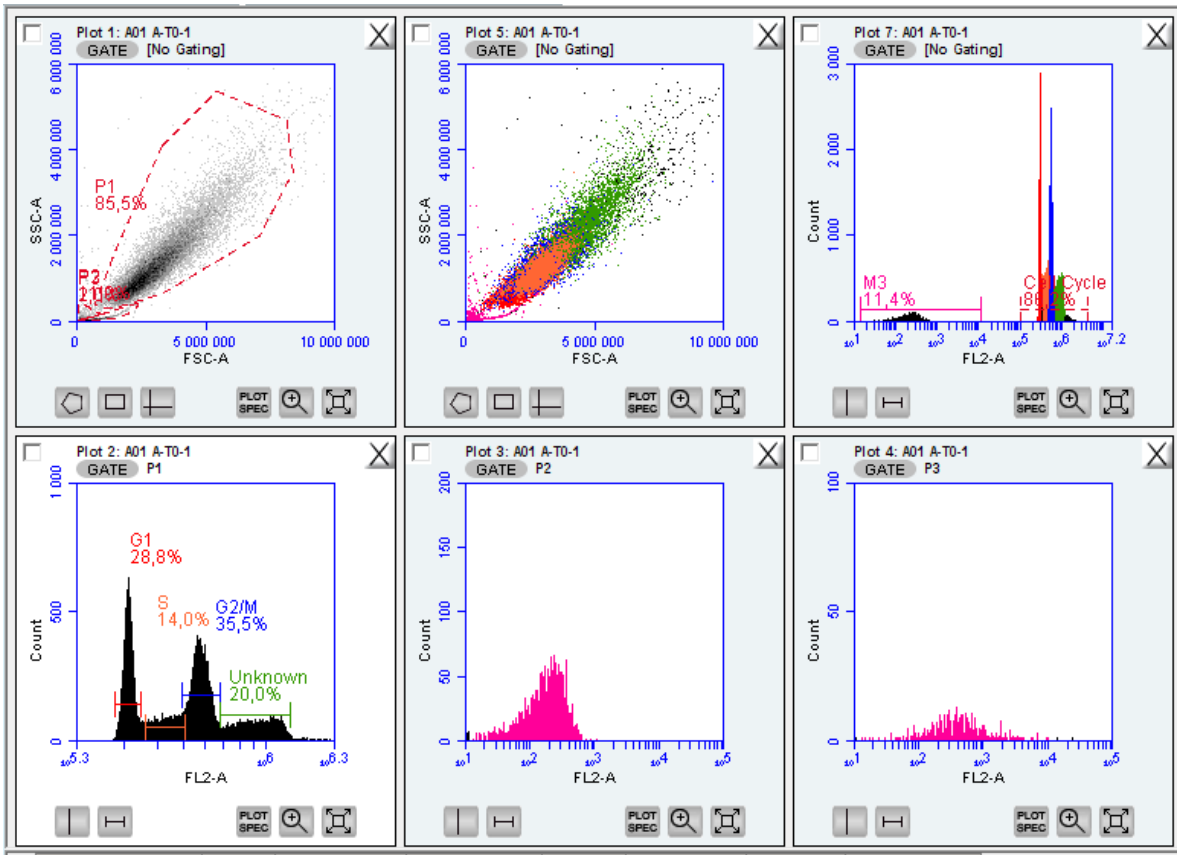
Plot 1: D06 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FSC-A	Mean SSC-A	CV FSC-A	CV SSC-A
All	10 000	372	100,00%	100,00%	1 755 662,19	1 170 778,13	167,93%	268,53%
P1	4 495	372	44,95%	44,95%	2 059 380,39	877 888,55	44,53%	50,82%
P2	1 464	372	14,64%	14,64%	518 006,79	35 681,98	79,87%	117,36%
P3	1 033	372	10,33%	10,33%	126 146,02	50 609,76	73,72%	84,35%

Plot 3: D06 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	372	100,00%	100,00%	138 507,44	140,85%	

Plot 2: D06 33.30G-24F-70E-2.00S-D+P	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
All	10 000	372	100,00%	100,00%	138 507,44	140,85%	

Figure 76. D-series sample Nr. 6. Stained with DAPI & PI. Prepared according to Appendix A39.

Appendix C1



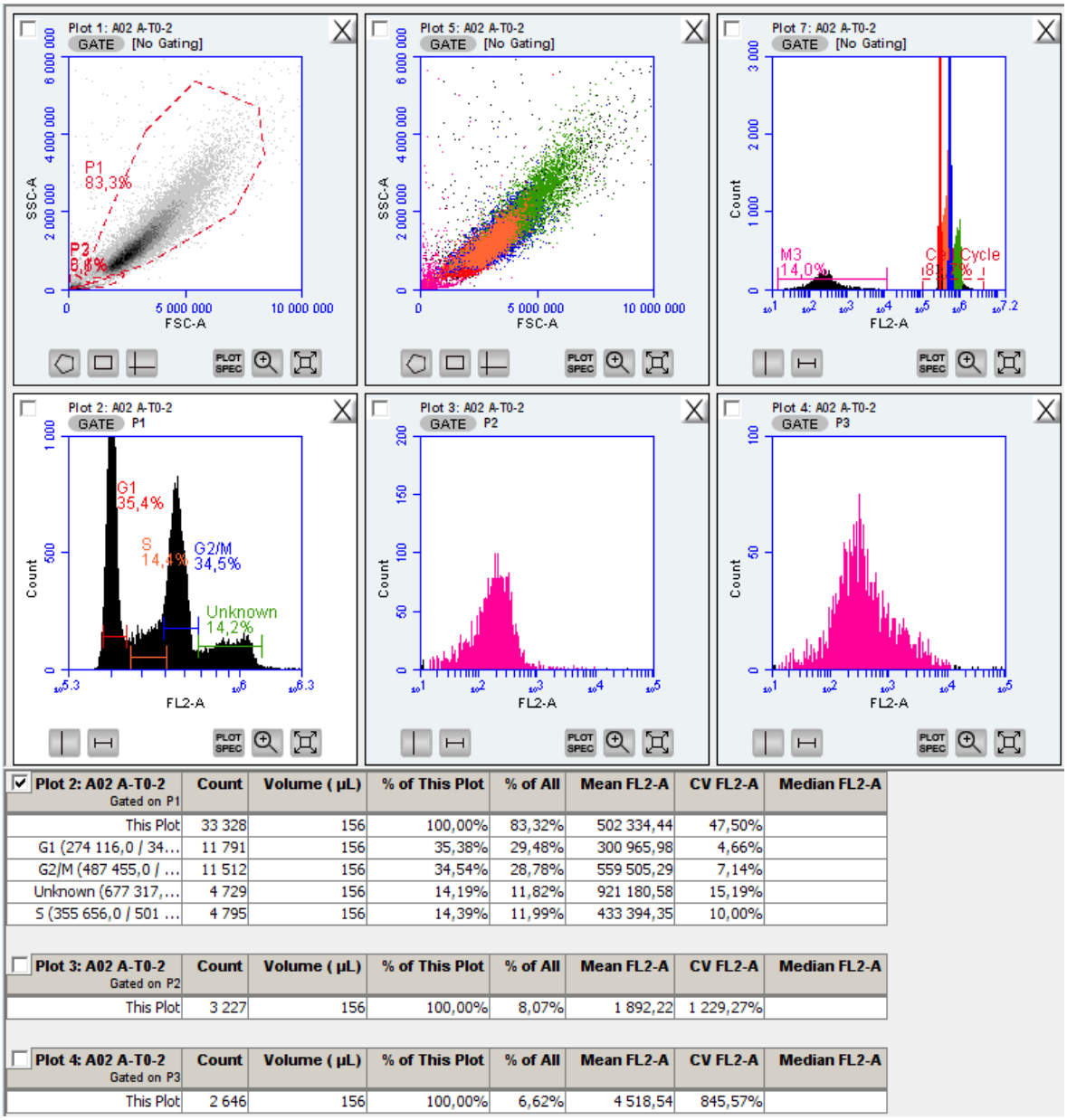
Plot 2: A01 A-T0-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	17 094	56	100,00%	85,47%	563 848,16	48,02%	
G1 (274 116,0 / 34...	4 927	56	28,82%	24,64%	309 822,56	4,31%	
G2/M (487 455,0 / ...	6 063	56	35,47%	30,31%	573 921,40	7,35%	
Unknown (677 317,...	3 419	56	20,00%	17,10%	934 317,70	16,14%	
S (355 656,0 / 501 ...	2 398	56	14,03%	11,99%	428 499,30	9,79%	

Plot 3: A01 A-T0-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 205	56	100,00%	11,02%	199,99	435,41%	

Plot 4: A01 A-T0-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	405	56	100,00%	2,02%	3 655,27	892,46%	

Figure 77. 20° C at 0 hours sample Nr. 1.

Appendix C2



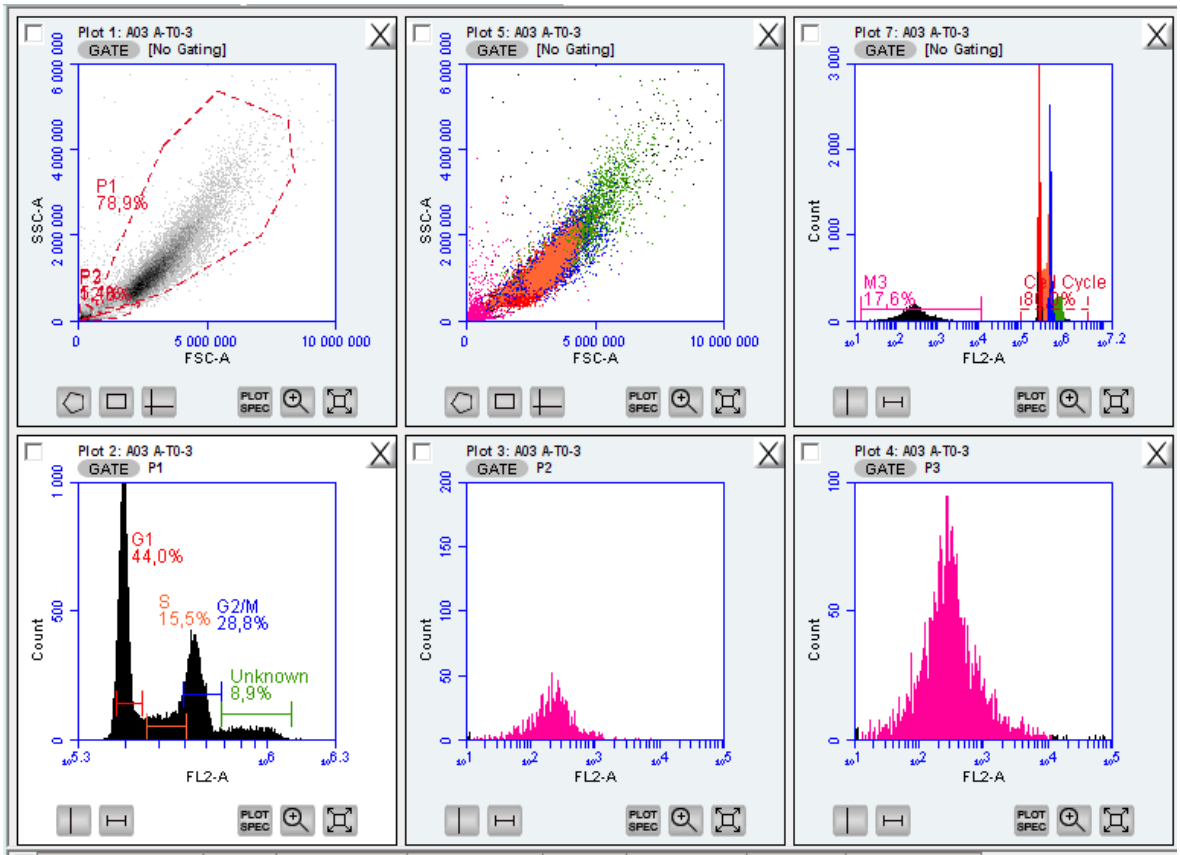
Plot 2: A02 A-T0-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	33 328	156	100,00%	83,32%	502 334,44	47,50%	
G1 (274 116,0 / 34...	11 791	156	35,38%	29,48%	300 965,98	4,66%	
G2/M (487 455,0 / ...	11 512	156	34,54%	28,78%	559 505,29	7,14%	
Unknown (677 317,...	4 729	156	14,19%	11,82%	921 180,58	15,19%	
S (355 656,0 / 501 ...	4 795	156	14,39%	11,99%	433 394,35	10,00%	

Plot 3: A02 A-T0-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 227	156	100,00%	8,07%	1 892,22	1 229,27%	

Plot 4: A02 A-T0-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 646	156	100,00%	6,62%	4 518,54	845,57%	

Figure 78. 20 °C at 0 hours sample Nr. 2.

Appendix C3



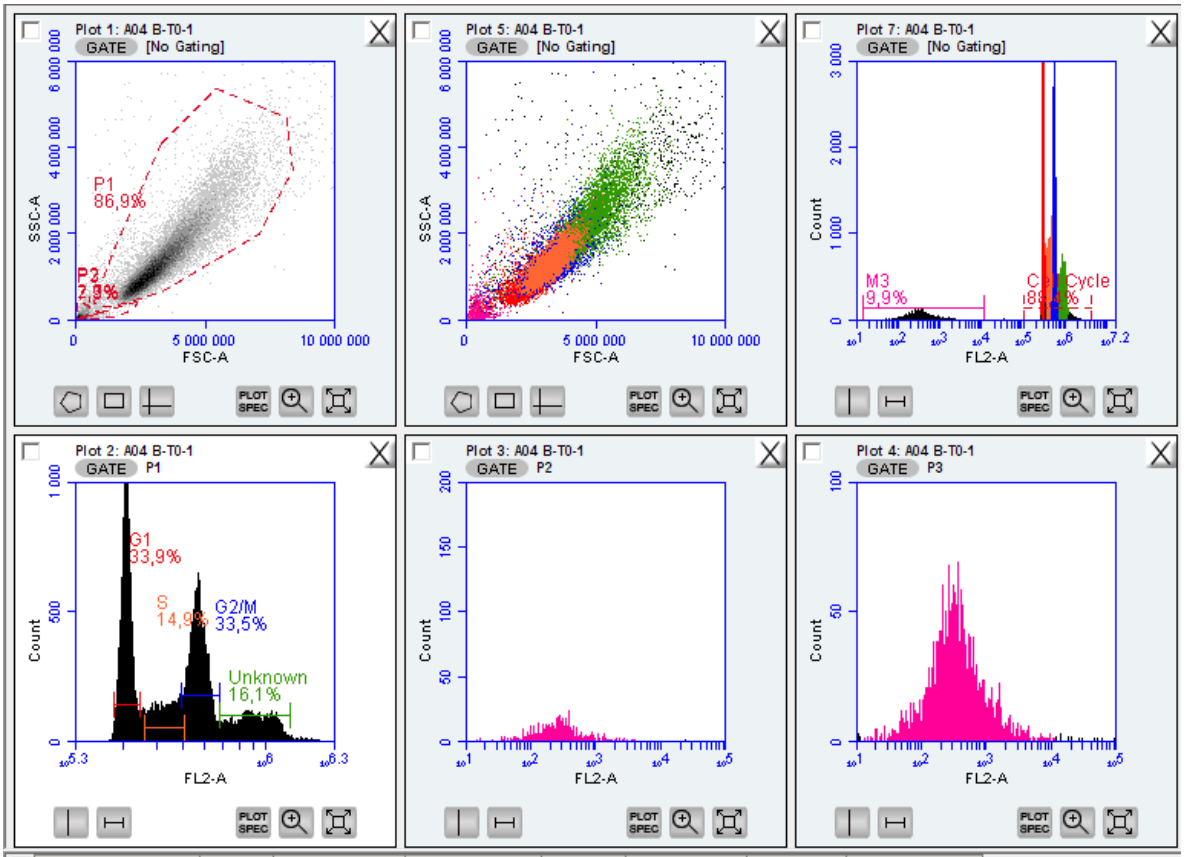
Plot 2: A03 A-T0-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	19 318	398	100,00%	78,92%	442 315,02	44,91%	
G1 (274 116,0 / 34...	8 509	398	44,05%	34,76%	296 552,05	4,72%	
G2/M (487 455,0 / ...	5 573	398	28,85%	22,77%	549 927,01	7,08%	
Unknown (677 317,...	1 722	398	8,91%	7,03%	888 911,33	14,86%	
S (355 656,0 / 501 ...	2 998	398	15,52%	12,25%	434 805,09	10,25%	

Plot 3: A03 A-T0-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 332	398	100,00%	5,44%	5 890,30	759,61%	

Plot 4: A03 A-T0-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 075	398	100,00%	12,56%	4 796,34	830,31%	

Figure 79. 20 °C at 0 hours sample Nr. 3.

Appendix C4



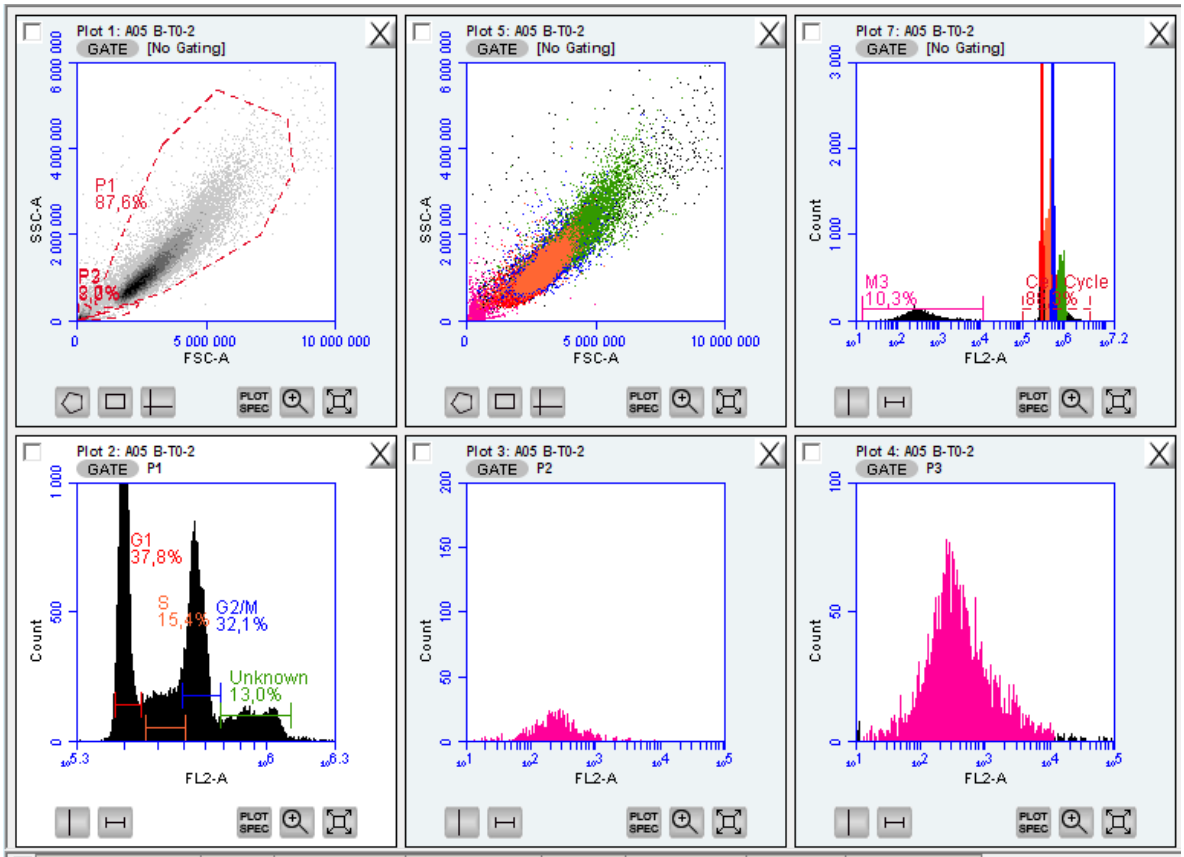
Plot 2: A04 B-T0-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	26 070	118	100,00%	86,90%	521 147,02	48,80%	
G1 (274 116,0 / 34...	8 835	118	33,89%	29,45%	306 823,38	4,52%	
G2/M (487 455,0 / ...	8 742	118	33,53%	29,14%	567 675,29	7,29%	
Unknown (677 317,...	4 204	118	16,13%	14,01%	916 315,00	15,58%	
S (355 656,0 / 501 ...	3 876	118	14,87%	12,92%	429 283,91	9,90%	

Plot 3: A04 B-T0-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	693	118	100,00%	2,31%	3 328,53	808,64%	

Plot 4: A04 B-T0-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 370	118	100,00%	7,90%	19 350,82	528,11%	

Figure 80. 26 °C at 0 hours sample Nr. 1.

Appendix C5



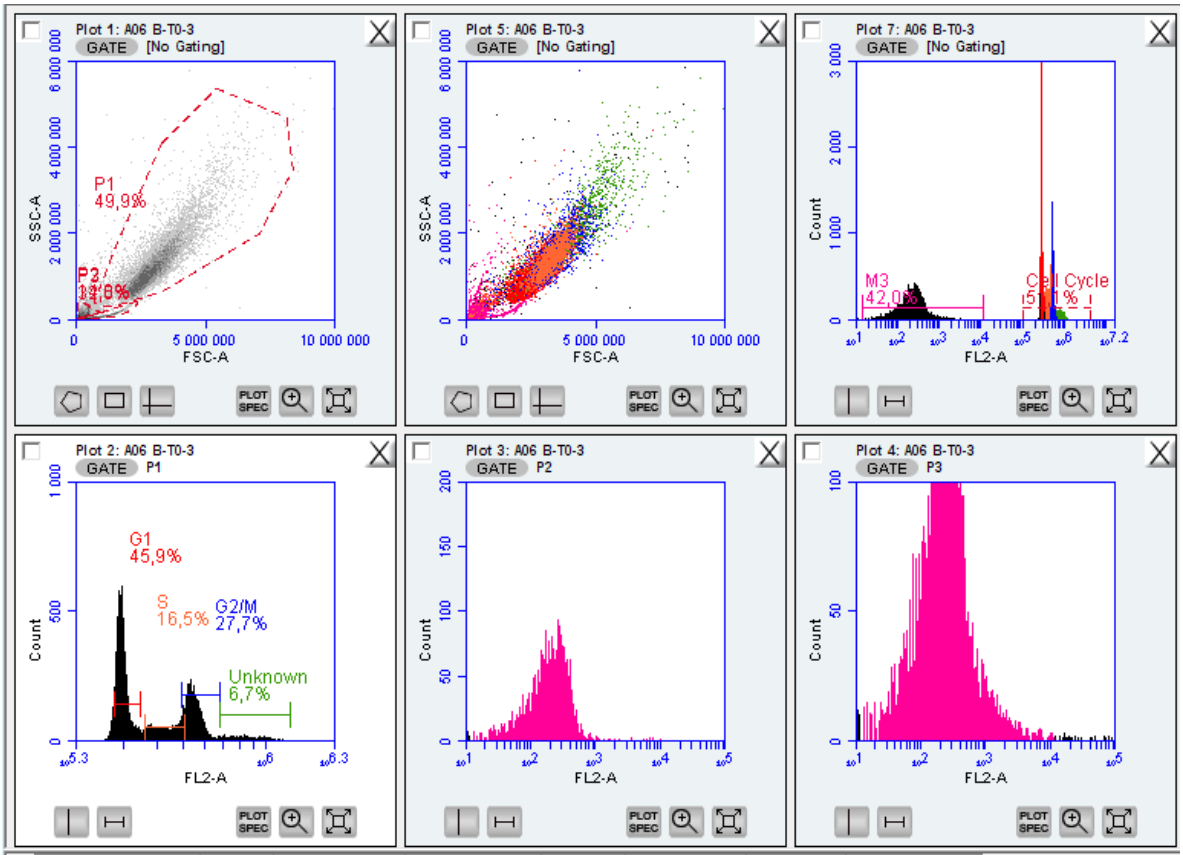
Plot 2: A05 B-T0-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	35 043	314	100,00%	87,59%	488 603,78	47,67%	
G1 (274 116,0 / 34…	13 235	314	37,77%	33,08%	299 893,37	4,61%	
G2/M (487 455,0 / …	11 233	314	32,05%	28,08%	557 134,57	7,06%	
Unknown (677 317,…	4 548	314	12,98%	11,37%	912 895,62	15,33%	
S (355 656,0 / 501 …	5 407	314	15,43%	13,51%	431 336,40	10,14%	

Plot 3: A05 B-T0-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	874	314	100,00%	2,18%	3 595,56	853,34%	

Plot 4: A05 B-T0-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 210	314	100,00%	8,02%	5 947,94	731,64%	

Figure 81. 26 °C at 0 hours sample Nr. 2.

Appendix C6



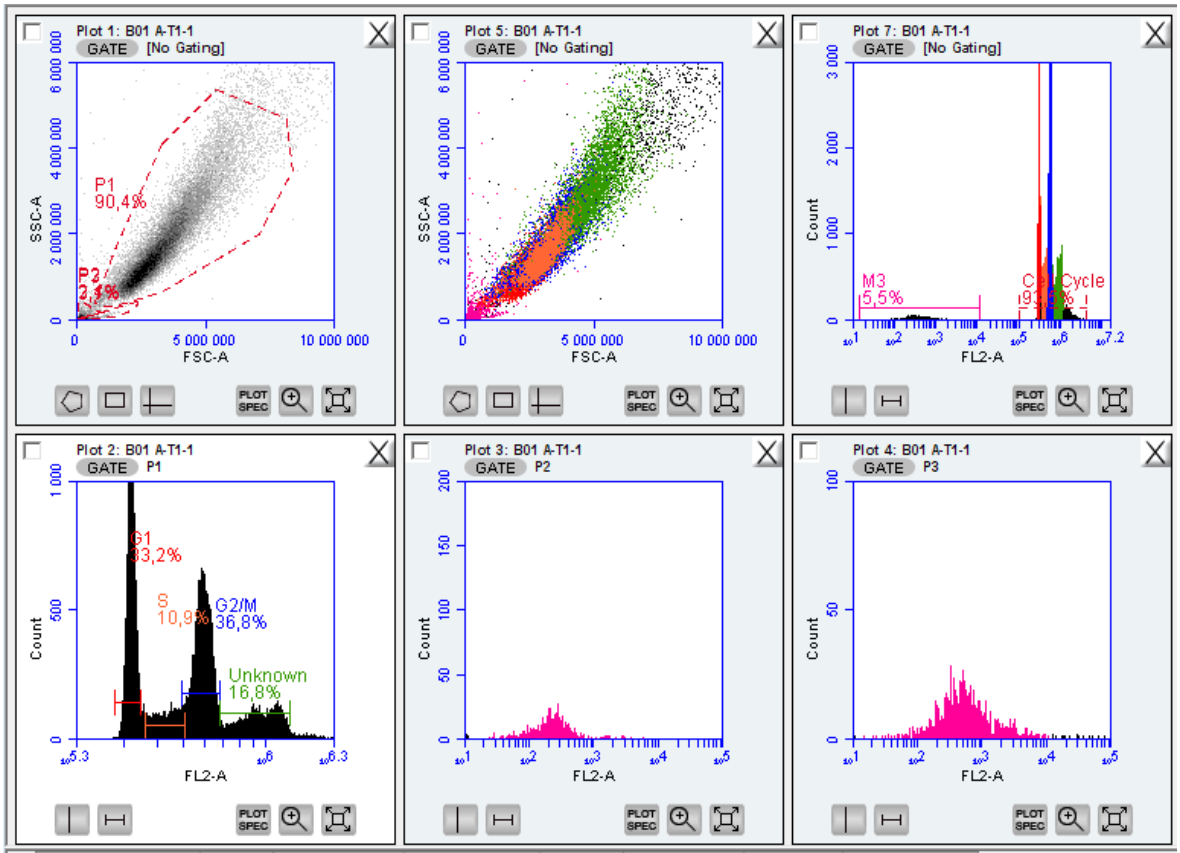
Plot 2: A06 B-T0-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	9 973	335	100,00%	49,92%	421 818,15	44,43%	
G1 (274 116,0 / 34…	4 582	335	45,94%	22,93%	295 616,40	4,86%	
G2/M (487 455,0 / …	2 767	335	27,74%	13,85%	545 259,86	6,81%	
Unknown (677 317,…	669	335	6,71%	3,35%	875 458,61	15,09%	
S (355 656,0 / 501 …	1 647	335	16,51%	8,24%	431 178,15	10,52%	

Plot 3: A06 B-T0-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 966	335	100,00%	14,85%	2 145,97	1 478,35%	

Plot 4: A06 B-T0-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	6 386	335	100,00%	31,96%	8 147,47	700,00%	

Figure 82. 26 °C at 0 hours sample Nr. 3.

Appendix C7



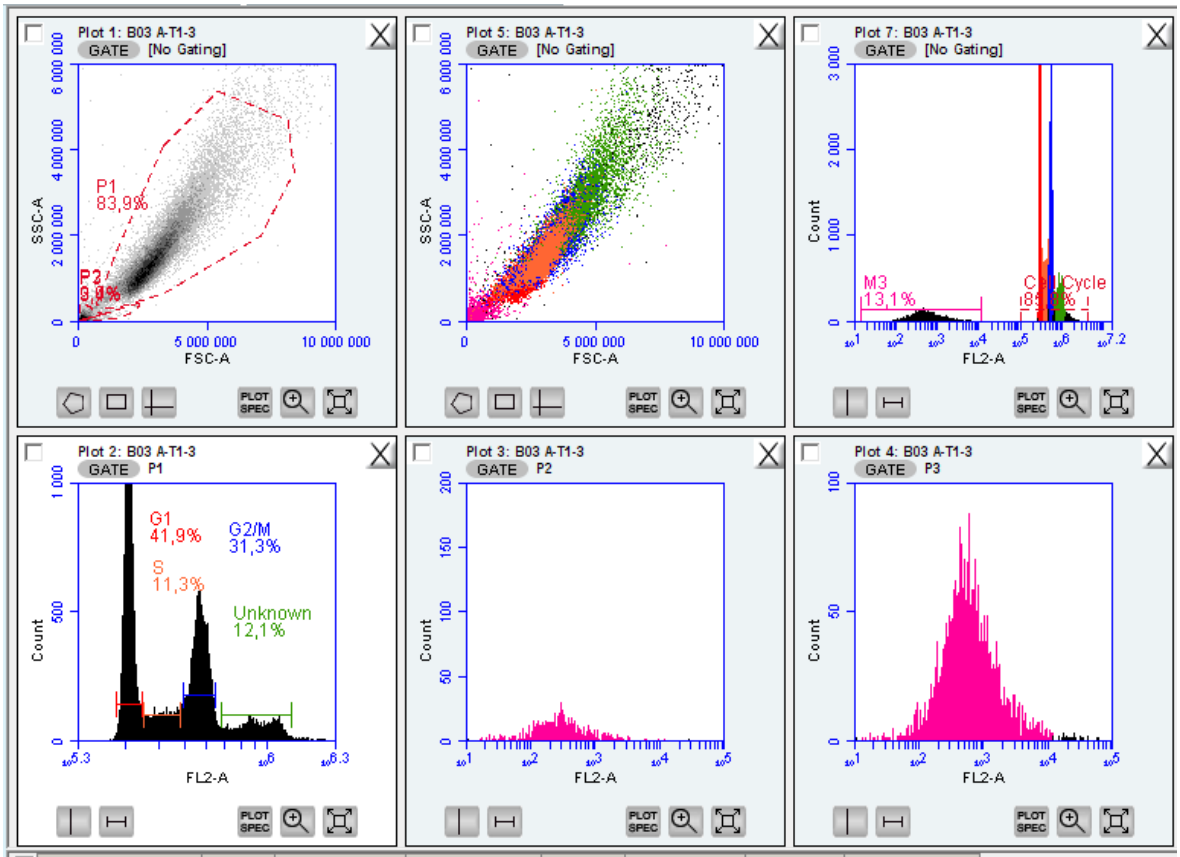
Plot 2: B01 A-T1-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	26 093	199	100,00%	90,40%	554 231,06	48,04%	
G1 (274 116,0 / 34...	8 662	199	33,20%	30,01%	317 406,06	3,68%	
G2/M (487 455,0 / ...	9 603	199	36,80%	33,27%	584 815,04	7,20%	
Unknown (677 317,...	4 391	199	16,83%	15,21%	955 443,57	15,73%	
S (355 656,0 / 501 ...	2 851	199	10,93%	9,88%	428 448,52	9,95%	

Plot 3: B01 A-T1-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	696	199	100,00%	2,41%	1 115,76	950,82%	

Plot 4: B01 A-T1-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	883	199	100,00%	3,06%	17 459,73	472,21%	

Figure 83. 20 °C at 2 hours sample Nr. 1.

Appendix C8



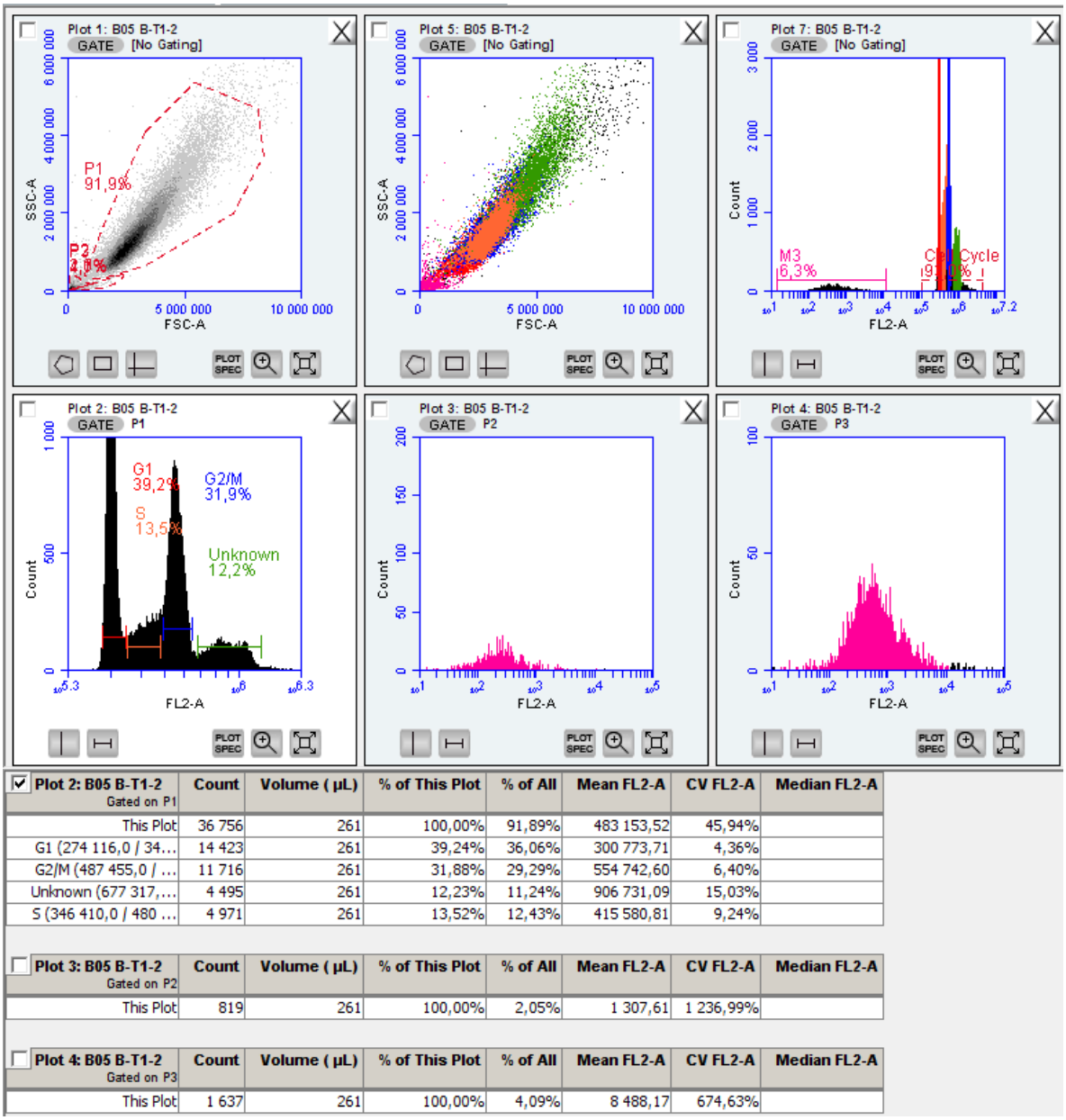
Plot 2: B03 A-T1-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	24 749	264	100,00%	83,93%	489 913,73	47,72%	
G1 (274 116,0 / 34...	10 361	264	41,86%	35,14%	307 593,05	4,02%	
G2/M (487 455,0 / ...	7 741	264	31,28%	26,25%	566 442,42	6,54%	
Unknown (677 317,...	2 999	264	12,12%	10,17%	929 025,83	15,63%	
S (346 410,0 / 480 ...	2 806	264	11,34%	9,52%	411 885,43	9,50%	

Plot 3: B03 A-T1-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	891	264	100,00%	3,02%	1 578,50	1 412,17%	

Plot 4: B03 A-T1-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 762	264	100,00%	9,37%	8 448,32	654,45%	

Figure 84. 20 °C at 2 hours sample Nr. 3.

Appendix C9



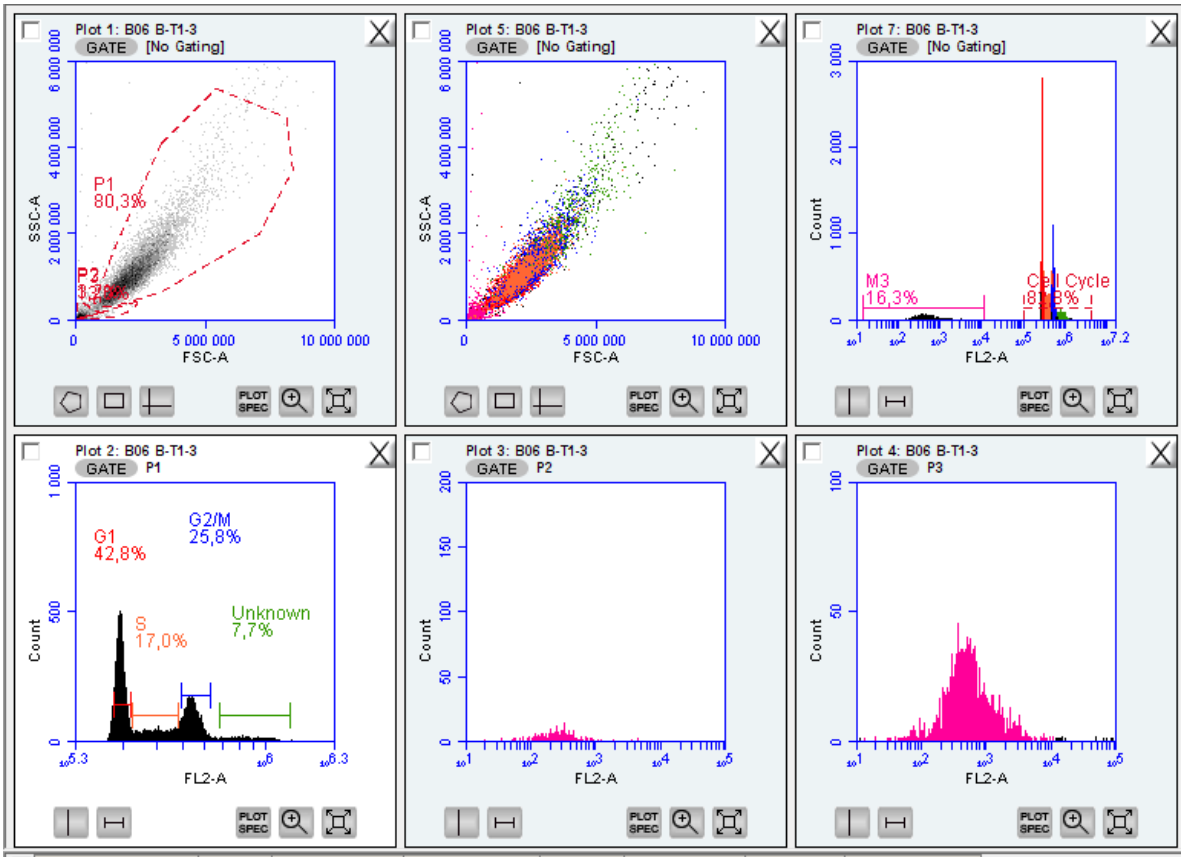
Plot 2: B05 B-T1-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	36 756	261	100,00%	91,89%	483 153,52	45,94%	
G1 (274 116,0 / 34...	14 423	261	39,24%	36,06%	300 773,71	4,36%	
G2/M (487 455,0 / ...	11 716	261	31,88%	29,29%	554 742,60	6,40%	
Unknown (677 317,...	4 495	261	12,23%	11,24%	906 731,09	15,03%	
S (346 410,0 / 480 ...	4 971	261	13,52%	12,43%	415 580,81	9,24%	

Plot 3: B05 B-T1-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	819	261	100,00%	2,05%	1 307,61	1 236,99%	

Plot 4: B05 B-T1-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 637	261	100,00%	4,09%	8 488,17	674,63%	

Figure 85. 26 °C at 2 hours sample Nr. 2.

Appendix C10



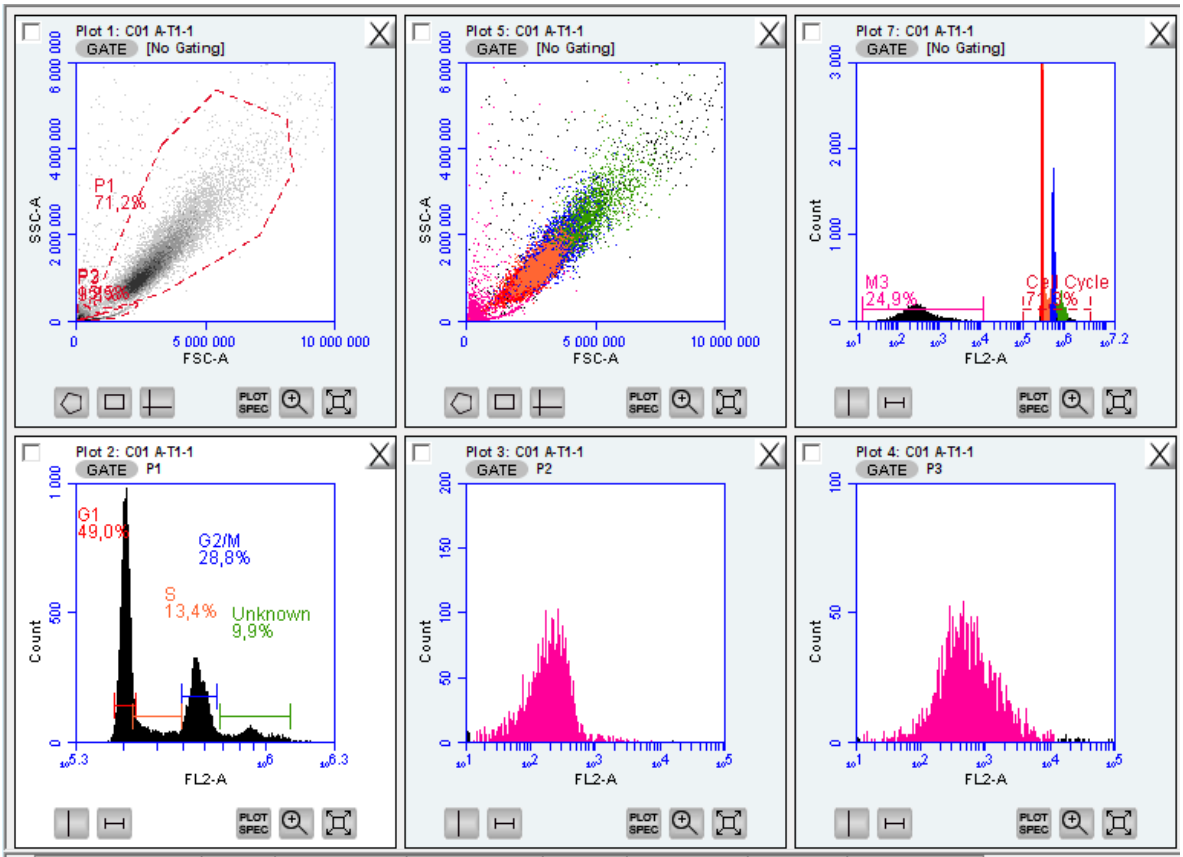
Plot 2: B06 B-T1-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	8 339	150	100,00%	80,34%	430 558,84	45,64%	
G1 (274 116,0 / 32...	3 572	150	42,83%	34,41%	292 148,23	3,44%	
G2/M (487 455,0 / ...	2 155	150	25,84%	20,76%	541 702,51	6,00%	
Unknown (677 317,...	638	150	7,65%	6,15%	885 633,31	15,33%	
S (320 266,0 / 480 ...	1 418	150	17,00%	13,66%	399 234,09	11,80%	

Plot 3: B06 B-T1-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	335	150	100,00%	3,23%	12 544,47	469,22%	

Plot 4: B06 B-T1-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 348	150	100,00%	12,99%	14 438,70	459,06%	

Figure 86. 26 °C at 2 hours sample Nr. 3.

Appendix C11



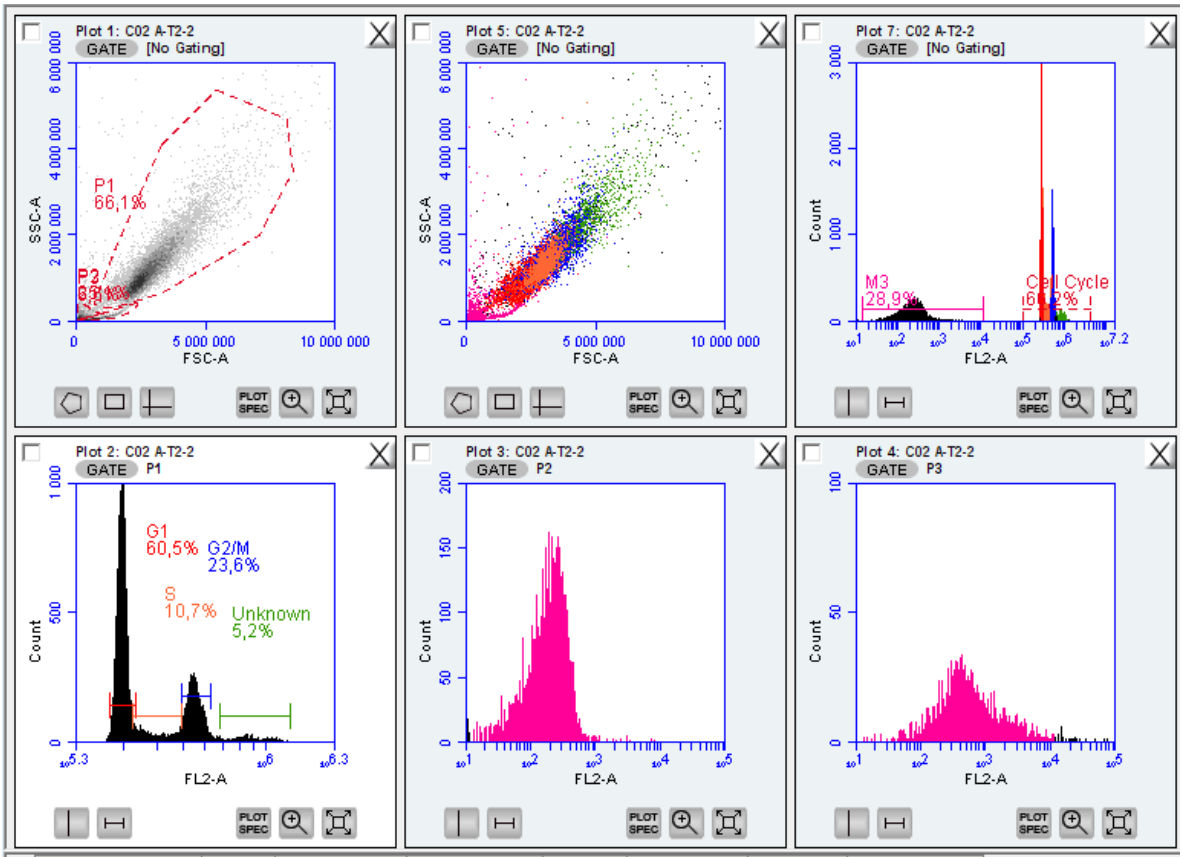
Plot 2: C01 A-T1-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	15 268	163	100,00%	71,20%	454 374,56	48,75%	
G1 (274 116,0 / 33...	7 486	163	49,03%	34,91%	303 346,90	3,82%	
G2/M (487 455,0 / ...	4 401	163	28,82%	20,52%	566 471,07	6,84%	
Unknown (677 317,...	1 507	163	9,87%	7,03%	908 770,00	15,73%	
S (320 266,0 / 493 ...	2 050	163	13,43%	9,56%	378 723,82	13,86%	

Plot 3: C01 A-T1-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 323	163	100,00%	15,50%	431,71	1 153,94%	

Plot 4: C01 A-T1-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 108	163	100,00%	9,83%	2 573,52	807,86%	

Figure 87. 20 °C at 4 hours sample Nr. 1.

Appendix C12



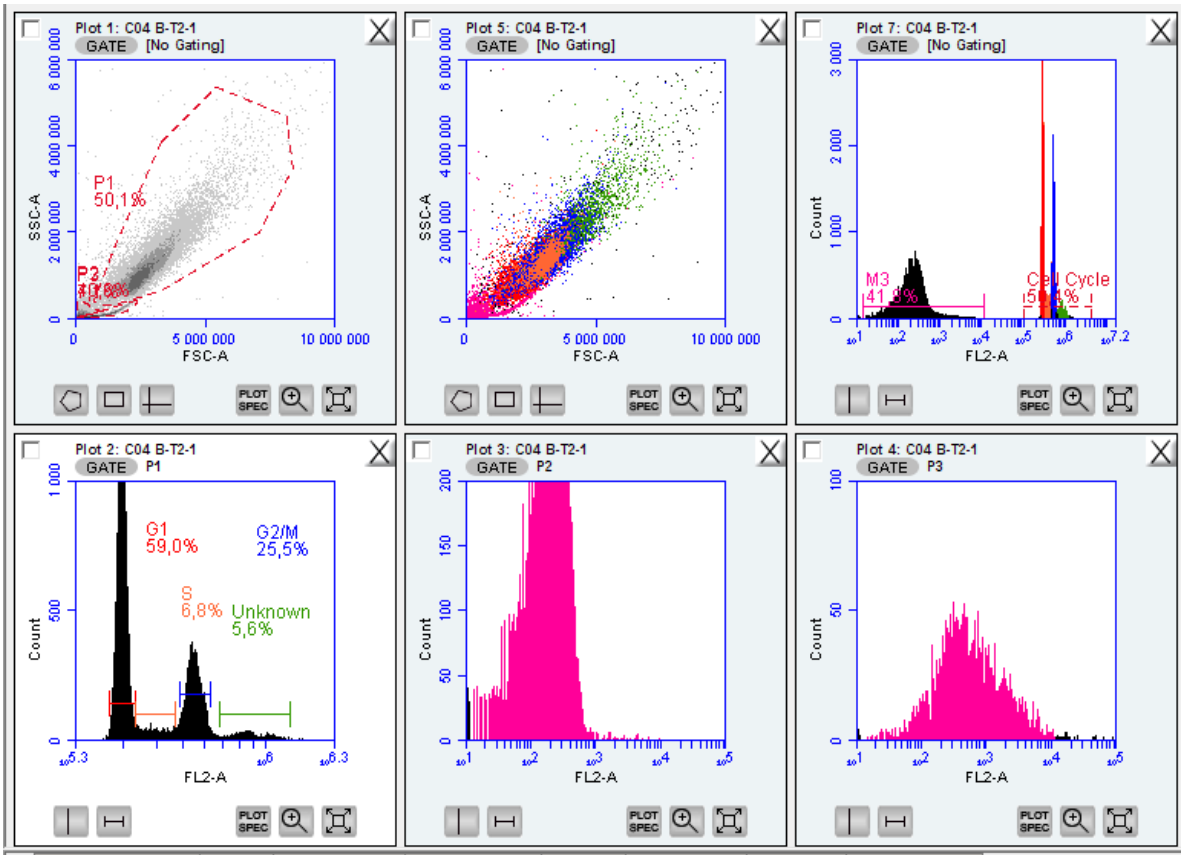
Plot 2: C02 A-T2-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	13 556	246	100,00%	66,08%	398 556,99	44,76%	
G1 (263 215,0 / 33…	8 198	246	60,48%	39,96%	294 316,28	4,18%	
G2/M (487 455,0 / …	3 197	246	23,58%	15,58%	548 029,05	5,95%	
Unknown (677 317,…	710	246	5,24%	3,46%	901 349,90	15,05%	
S (320 266,0 / 493 …	1 445	246	10,66%	7,04%	394 171,33	14,41%	

Plot 3: C02 A-T2-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 144	246	100,00%	25,07%	324,83	1 672,83%	

Plot 4: C02 A-T2-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 354	246	100,00%	6,60%	3 791,55	671,52%	

Figure 88. 20 °C at 4 hours sample Nr. 2.

Appendix C13



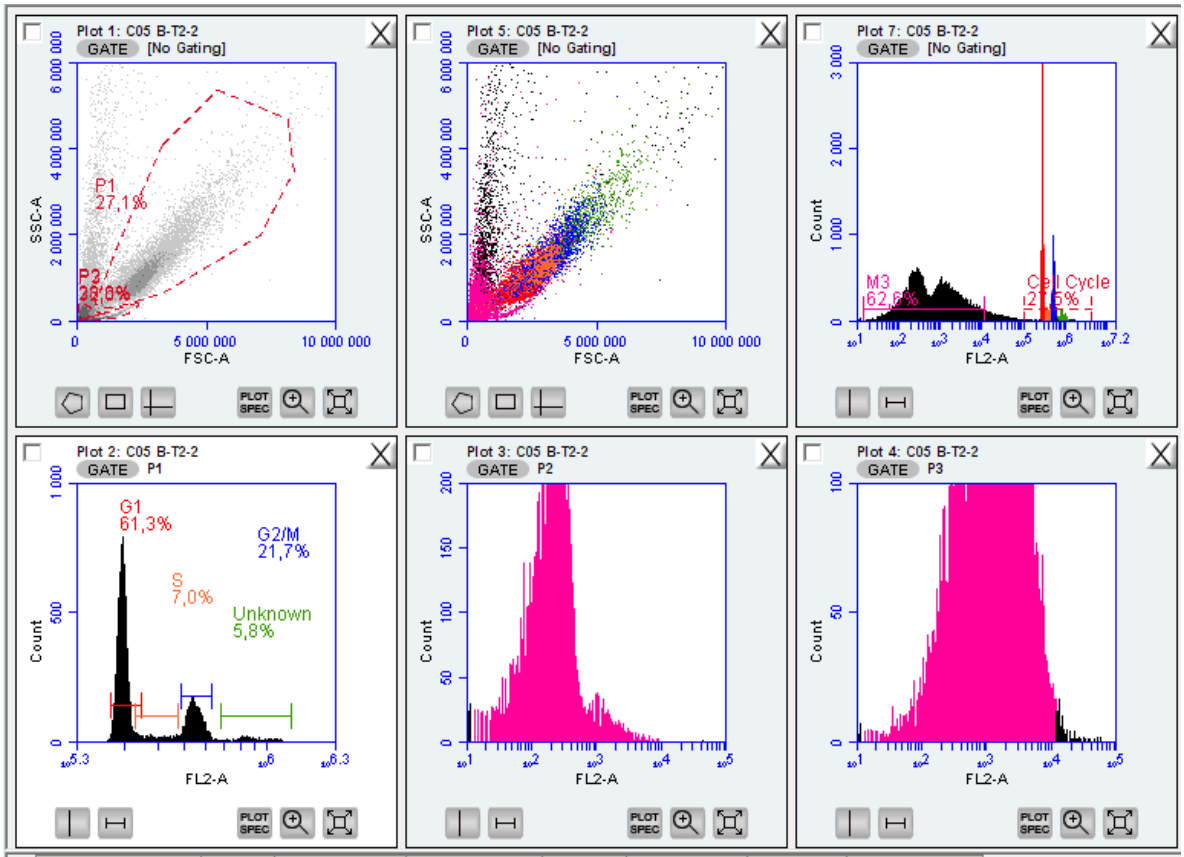
Plot 2: C04 B-T2-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	17 990	314	100,00%	50,08%	405 068,90	45,68%	
G1 (263 215,0 / 33...	10 620	314	59,03%	29,57%	295 144,99	4,08%	
G2/M (480 387,0 / ...	4 588	314	25,50%	12,77%	547 950,49	6,12%	
Unknown (677 317,...	1 005	314	5,59%	2,80%	906 497,04	15,04%	
S (328 754,0 / 467 ...	1 230	314	6,84%	3,42%	392 743,91	10,56%	

Plot 3: C04 B-T2-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	14 568	314	100,00%	40,56%	349,57	2 009,74%	

Plot 4: C04 B-T2-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 566	314	100,00%	7,14%	6 809,38	609,11%	

Figure 89. 26 °C at 4 hours sample Nr. 1.

Appendix C14



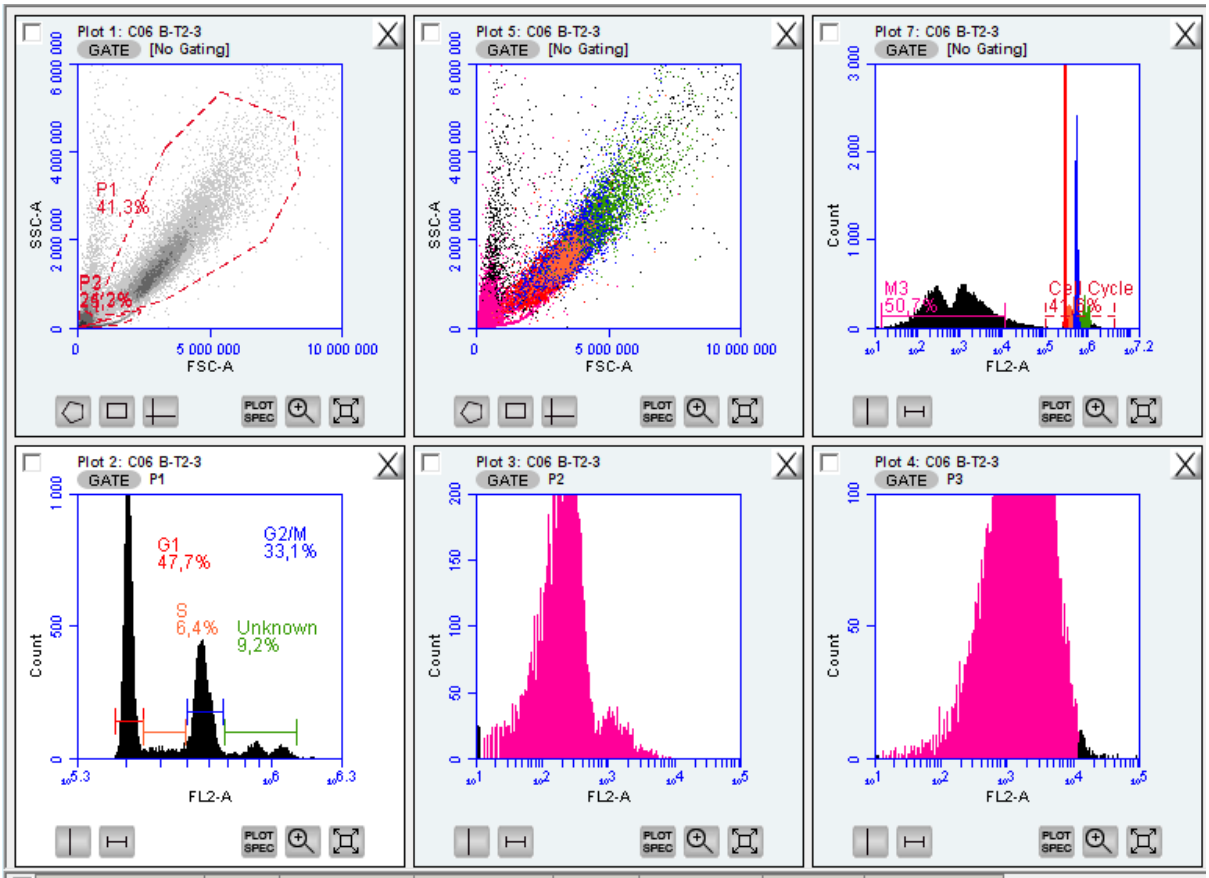
Plot 2: C05 B-T2-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	9 845	384	100,00%	27,08%	386 166,64	51,35%	
G1 (263 215,0 / 34...	6 033	384	61,28%	16,60%	293 316,44	4,39%	
G2/M (480 387,0 / ...	2 132	384	21,66%	5,86%	545 464,52	6,17%	
Unknown (677 317,...	568	384	5,77%	1,56%	911 969,38	14,82%	
S (324 482,0 / 474 ...	688	384	6,99%	1,89%	390 861,46	11,68%	

Plot 3: C05 B-T2-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	10 414	384	100,00%	28,65%	449,57	1 565,34%	

Plot 4: C05 B-T2-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	11 619	384	100,00%	31,96%	2 661,74	551,88%	

Figure 90. 26 °C at 4 hours sample Nr. 2.

Appendix C15



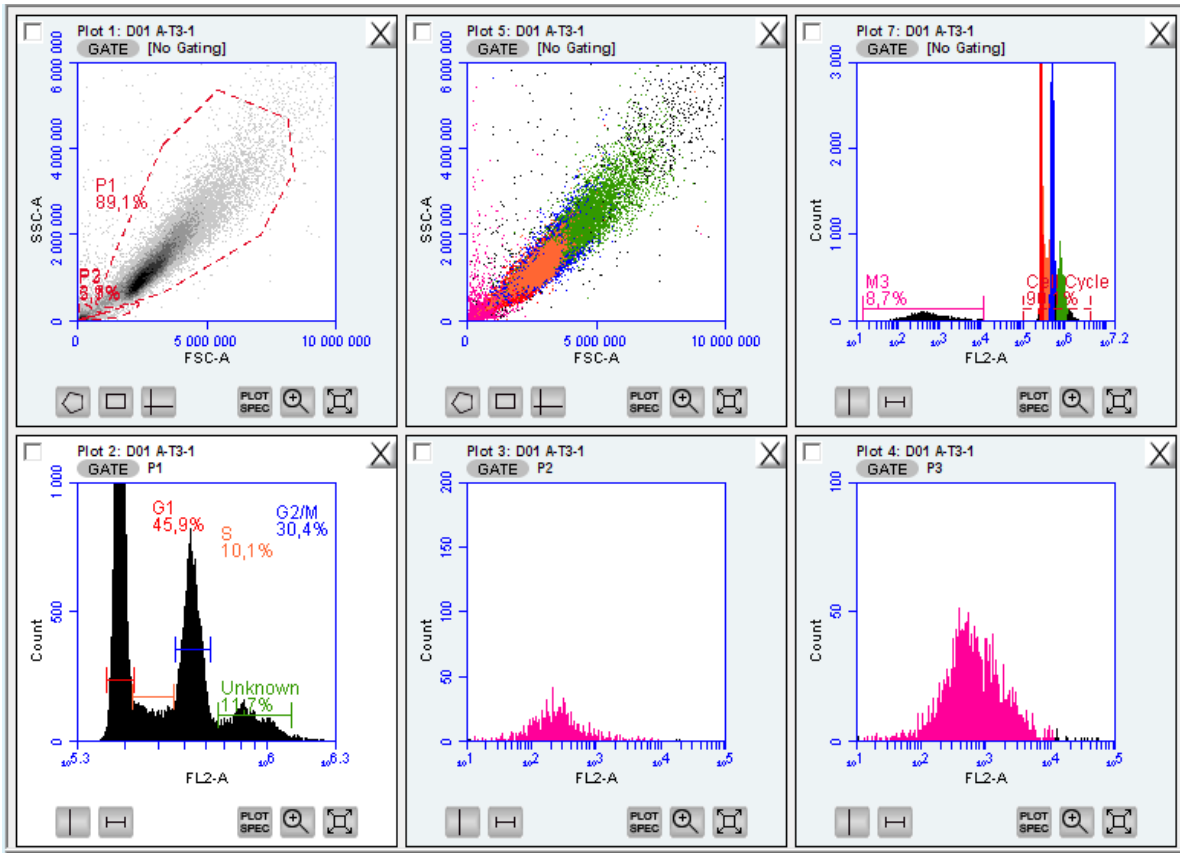
Plot 2: C06 B-T2-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	16 512	262	100,00%	41,28%	456 115,41	50,06%	
G1 (270 191,0 / 34...	7 875	262	47,69%	19,69%	304 119,35	3,72%	
G2/M (493 118,0 / ...	5 460	262	33,07%	13,65%	567 005,30	6,23%	
Unknown (677 317,...	1 516	262	9,18%	3,79%	937 887,97	15,31%	
S (341 909,0 / 493 ...	1 055	262	6,39%	2,64%	413 760,78	10,76%	

Plot 3: C06 B-T2-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	9 672	262	100,00%	24,18%	387,64	1 208,47%	

Plot 4: C06 B-T2-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	10 111	262	100,00%	25,28%	2 548,90	436,04%	

Figure 91. 26 °C at 4 hours sample Nr. 2.

Appendix C16



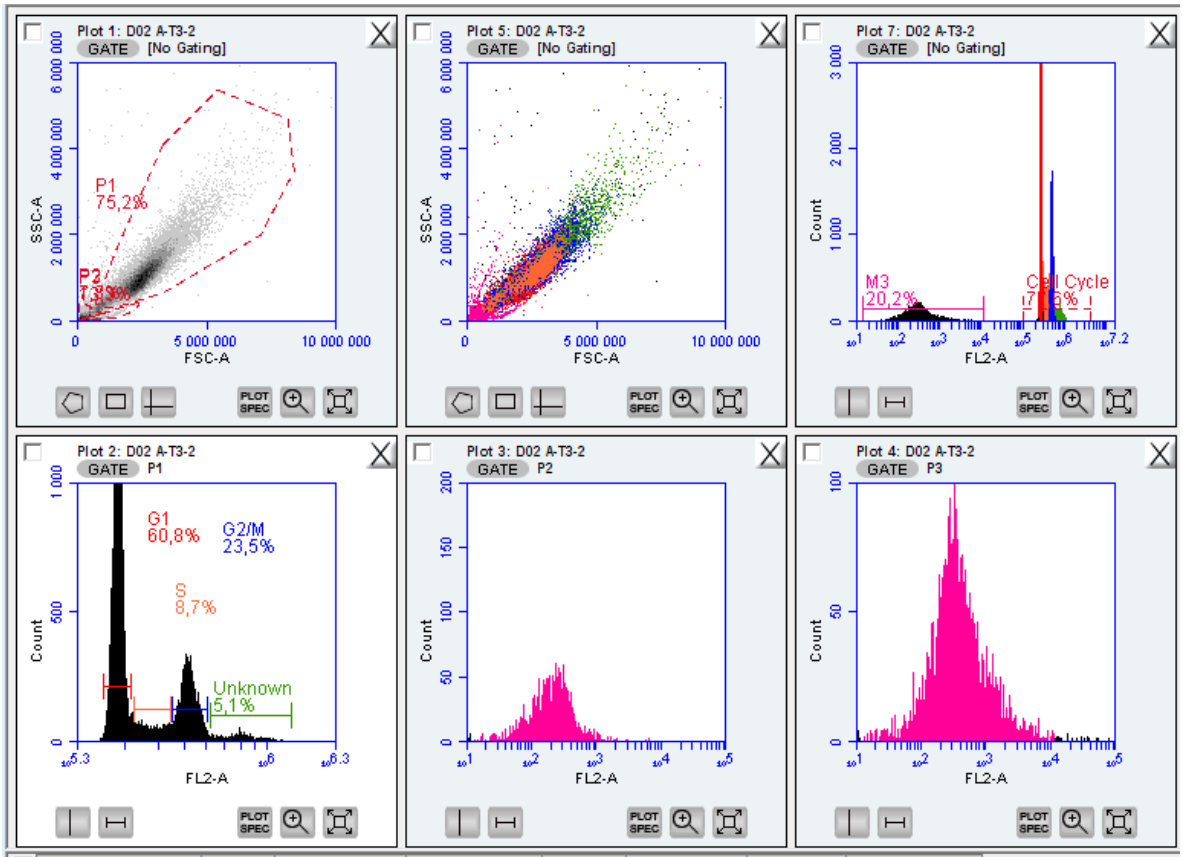
Plot 2: D01 A-T3-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	35 638	148	100,00%	89,10%	448 360,58	48,79%	
G1 (253 087,0 / 32...	16 368	148	45,93%	40,92%	286 496,05	4,38%	
G2/M (455 901,0 / ...	10 827	148	30,38%	27,07%	530 810,16	7,00%	
Unknown (657 524,...	4 158	148	11,67%	10,40%	876 242,51	15,62%	
S (316 104,0 / 455 ...	3 605	148	10,12%	9,01%	379 074,01	11,04%	

Plot 3: D01 A-T3-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 146	148	100,00%	2,86%	1 112,03	883,61%	

Plot 4: D01 A-T3-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 040	148	100,00%	5,10%	11 534,92	458,93%	

Figure 92. 20 °C at 6 hours sample Nr. 1.

Appendix C17



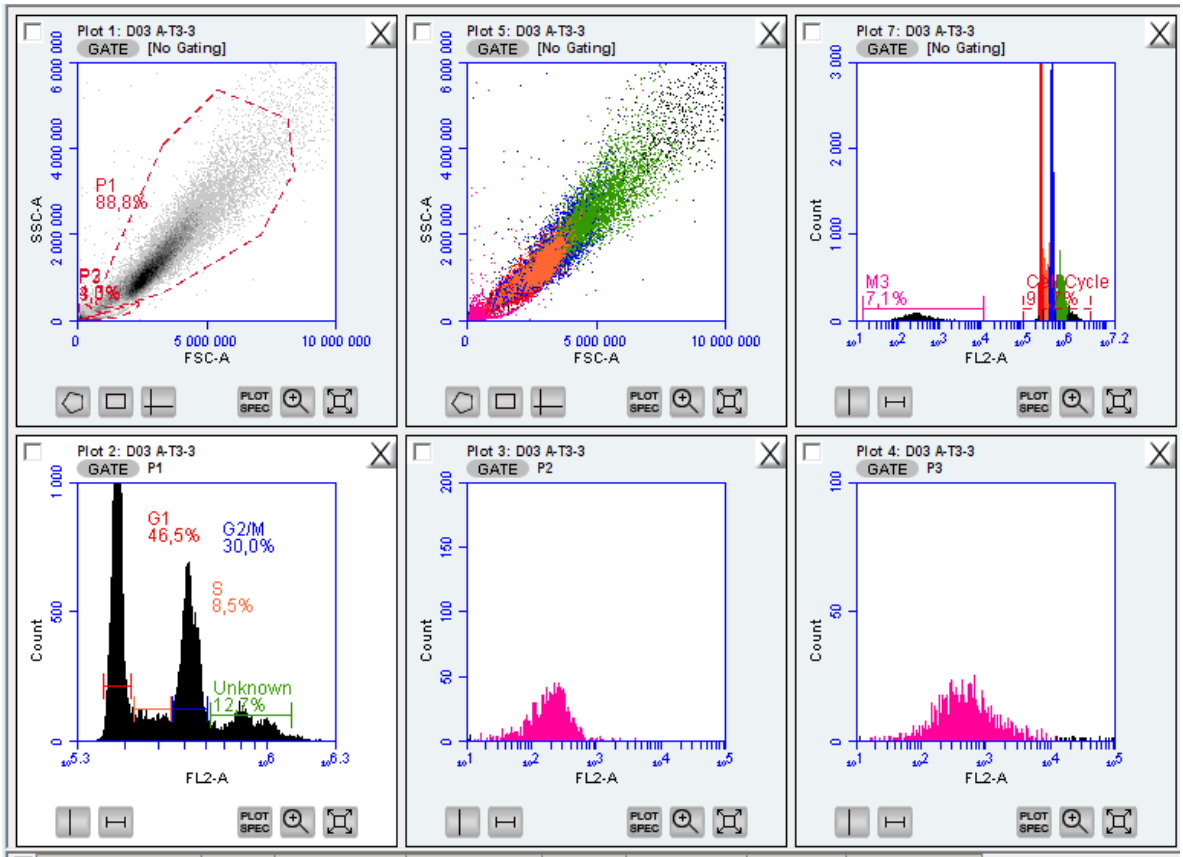
Plot 2: D02 A-T3-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	19 062	256	100,00%	75,17%	373 441,79	42,52%	
G1 (246 553,0 / 31…	11 589	256	60,80%	45,70%	280 823,27	4,23%	
G2/M (444 131,0 / …	4 475	256	23,48%	17,65%	516 409,13	6,90%	
Unknown (615 902,…	981	256	5,15%	3,87%	817 367,06	16,73%	
S (320 266,0 / 444 …	1 656	256	8,69%	6,53%	376 277,14	9,87%	

Plot 3: D02 A-T3-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 983	256	100,00%	7,82%	1 643,34	1 398,26%	

Plot 4: D02 A-T3-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 363	256	100,00%	13,26%	17 082,14	381,26%	

Figure 93. 20 °C at 6 hours sample Nr. 1.

Appendix C18



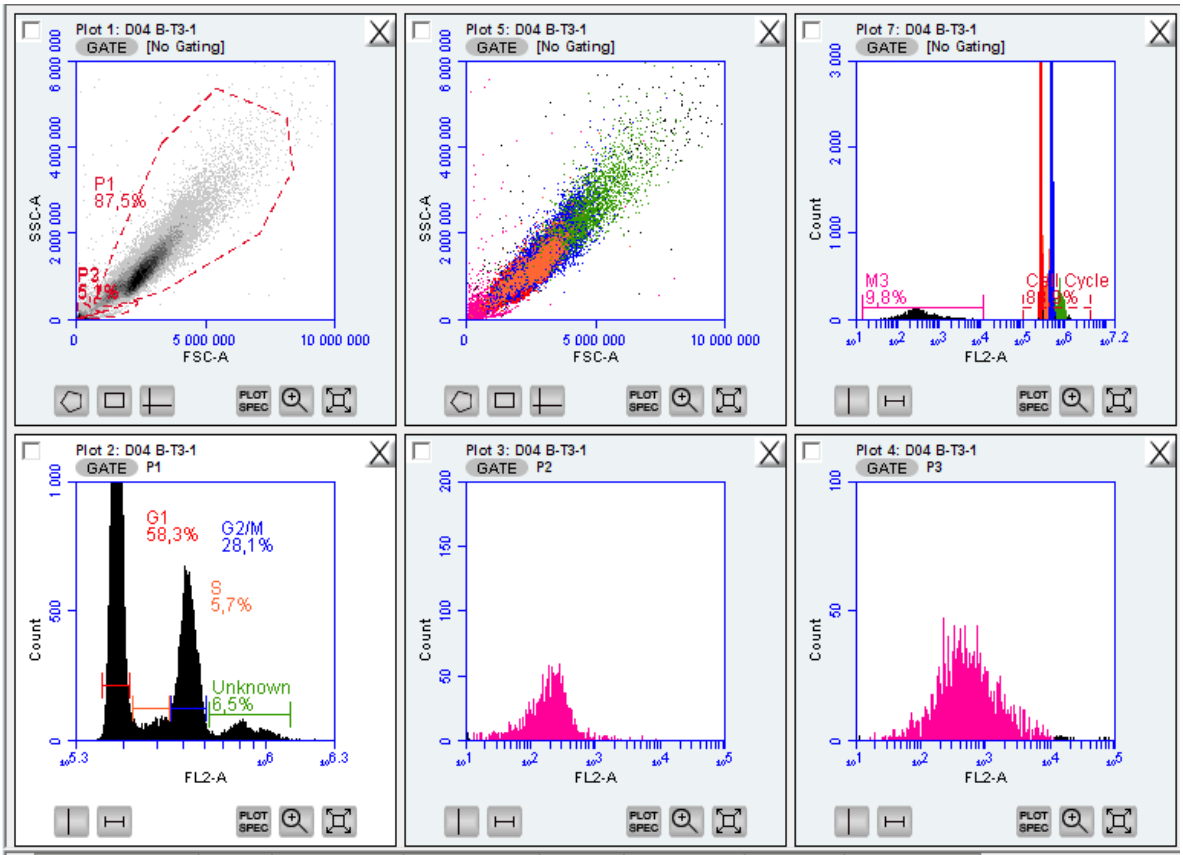
Plot 2: D03 A-T3-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	30 200	262	100,00%	88,79%	443 247,22	49,90%	
G1 (246 553,0 / 31…	14 043	262	46,50%	41,29%	279 445,49	4,10%	
G2/M (444 131,0 / …	9 060	262	30,00%	26,64%	519 347,58	6,85%	
Unknown (615 902,…	3 849	262	12,75%	11,32%	860 106,64	17,18%	
S (320 266,0 / 444 …	2 581	262	8,55%	7,59%	377 244,94	9,70%	

Plot 3: D03 A-T3-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 476	262	100,00%	4,34%	1 736,47	1 303,79%	

Plot 4: D03 A-T3-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 029	262	100,00%	3,03%	17 121,95	369,33%	

Figure 94. 20 °C at 6 hours sample Nr. 1.

Appendix C19



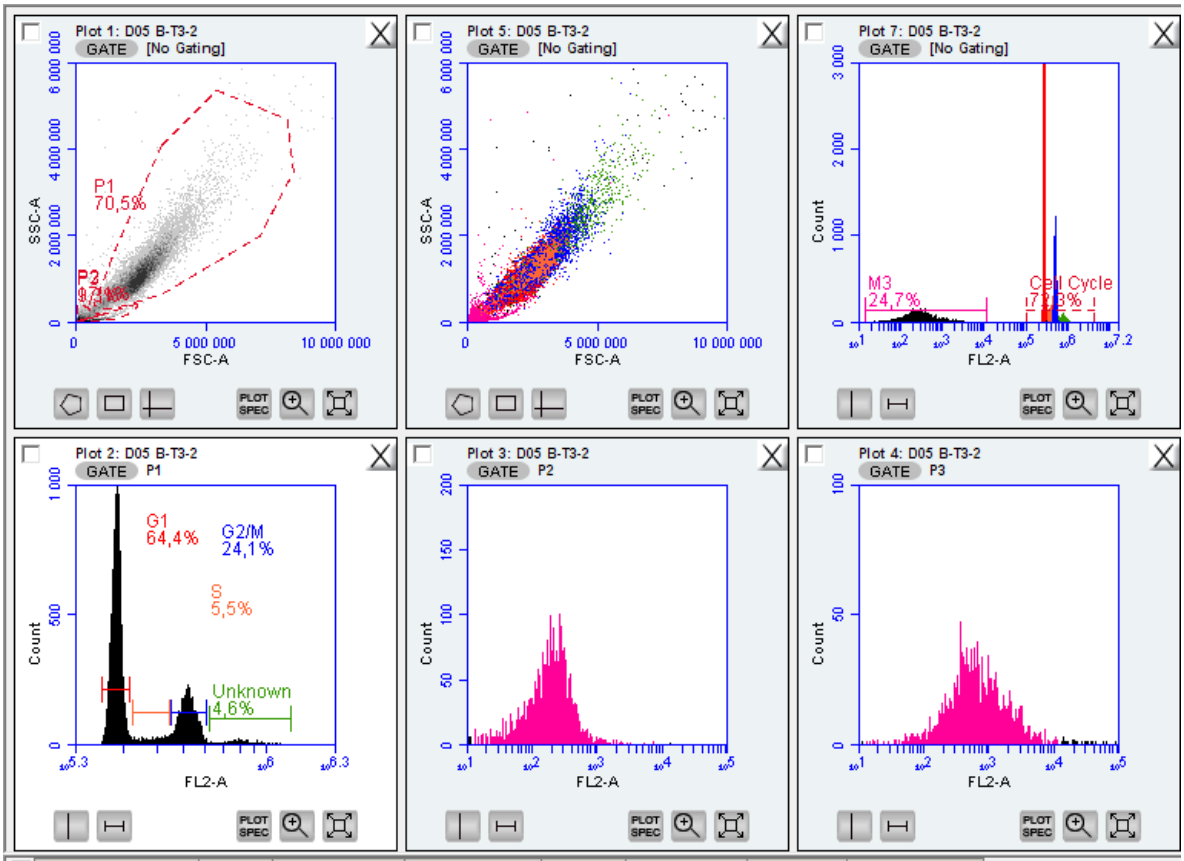
Plot 2: D04 B-T3-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	32 043	279	100,00%	87,52%	394 540,19	44,85%	
G1 (246 553,0 / 31…	18 675	279	58,28%	51,01%	281 875,77	4,18%	
G2/M (444 131,0 / …	9 015	279	28,13%	24,62%	519 238,15	6,74%	
Unknown (615 902,…	2 092	279	6,53%	5,71%	859 580,02	16,31%	
S (320 266,0 / 444 …	1 828	279	5,70%	4,99%	382 304,65	9,64%	

Plot 3: D04 B-T3-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 855	279	100,00%	5,07%	2 038,42	1 437,82%	

Plot 4: D04 B-T3-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 911	279	100,00%	5,22%	23 175,80	338,05%	

Figure 95. 26 °C at 6 hours sample Nr. 1.

Appendix C20



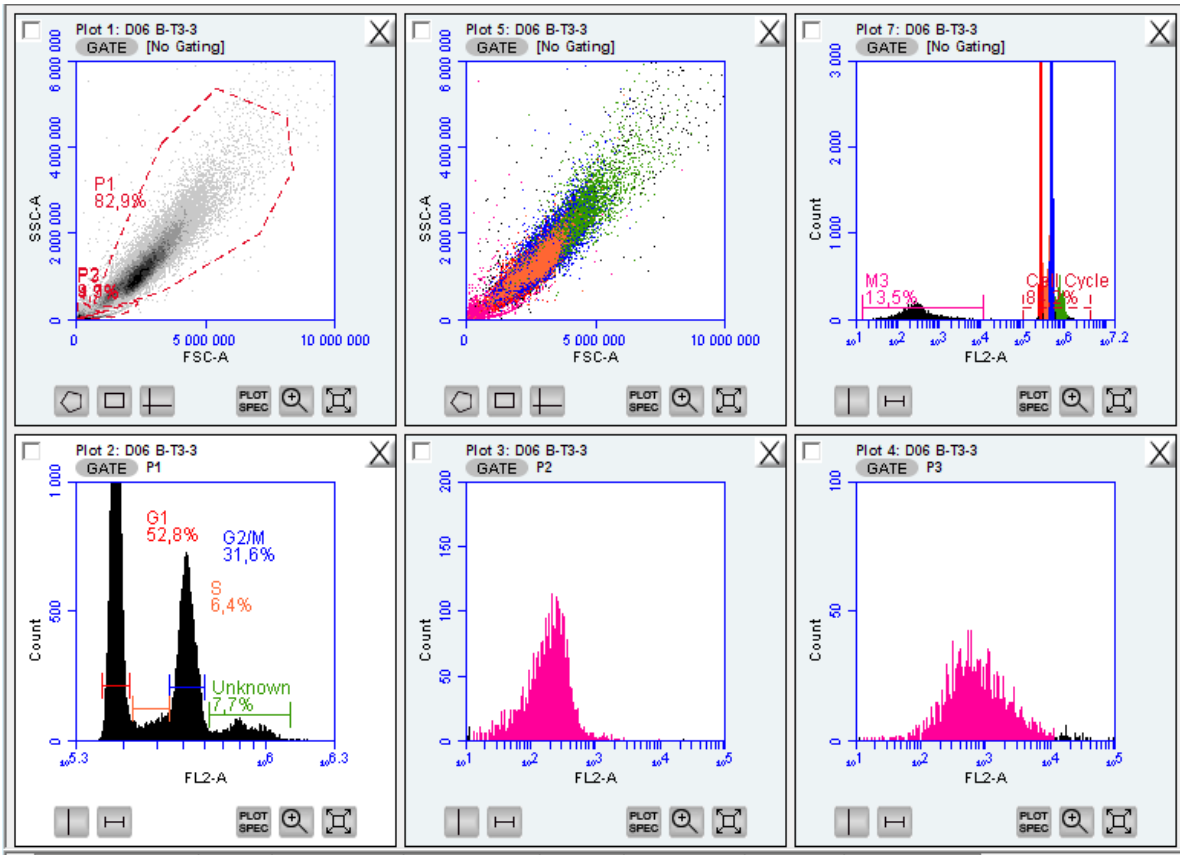
Plot 2: D05 B-T3-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	12 160	265	100,00%	70,51%	369 167,26	42,88%	
G1 (246 553,0 / 31…	7 828	265	64,38%	45,39%	280 651,65	4,05%	
G2/M (444 131,0 / …	2 934	265	24,13%	17,01%	517 275,17	6,73%	
Unknown (615 902,…	556	265	4,57%	3,22%	835 944,88	16,60%	
S (320 266,0 / 444 …	669	265	5,50%	3,88%	383 433,74	9,61%	

Plot 3: D05 B-T3-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 942	265	100,00%	17,06%	813,20	1 666,56%	

Plot 4: D05 B-T3-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 610	265	100,00%	9,34%	26 353,34	310,94%	

Figure 96. 26 °C at 6 hours sample Nr. 2.

Appendix C21



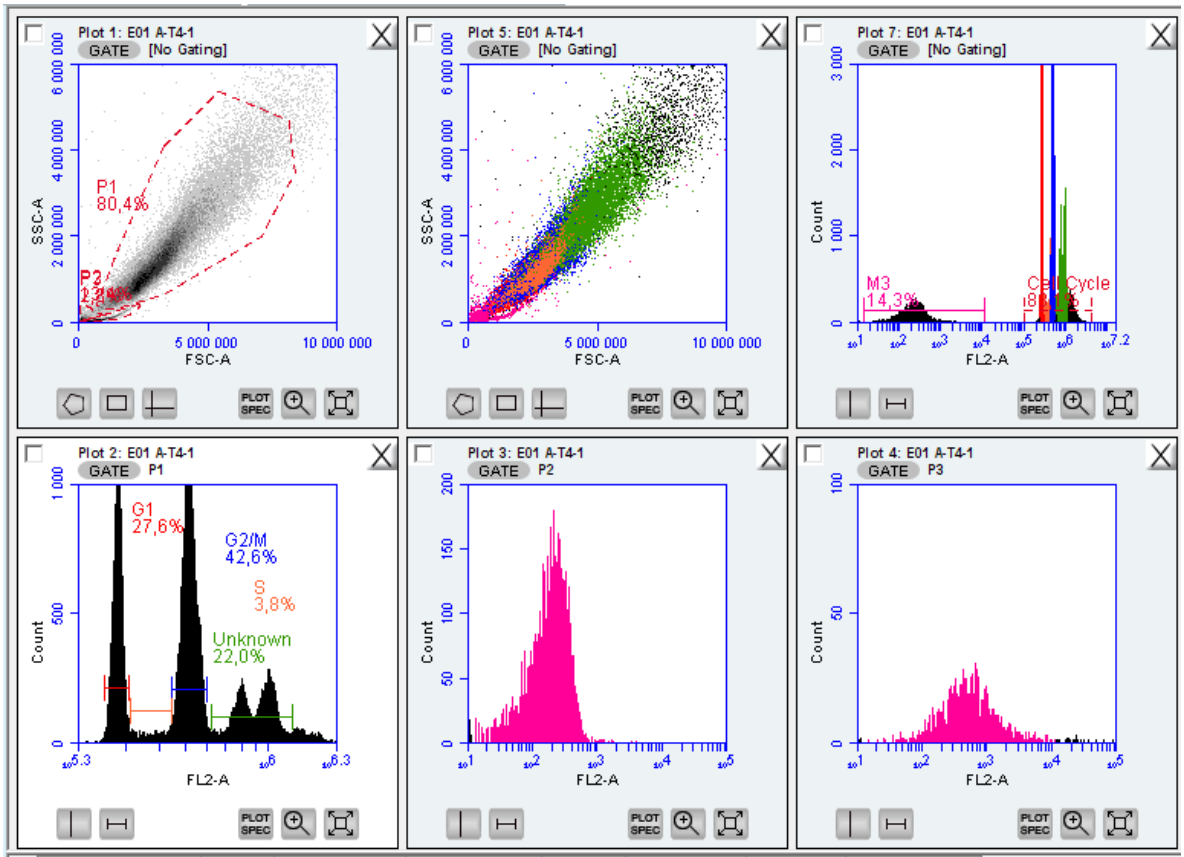
Plot 2: D06 B-T3-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	30 232	304	100,00%	82,95%	407 417,74	45,38%	
G1 (246 553,0 / 31…	15 965	304	52,81%	43,80%	279 605,85	4,14%	
G2/M (438 360,0 / …	9 560	304	31,62%	26,23%	513 589,83	6,67%	
Unknown (615 902,…	2 324	304	7,69%	6,38%	847 713,48	16,27%	
S (320 266,0 / 444 …	1 949	304	6,45%	5,35%	385 452,95	9,55%	

Plot 3: D06 B-T3-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 591	304	100,00%	9,85%	1 422,73	1 405,71%	

Plot 4: D06 B-T3-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 721	304	100,00%	4,72%	21 391,02	337,19%	

Figure 97. 26 °C at 6 hours sample Nr. 3.

Appendix C22



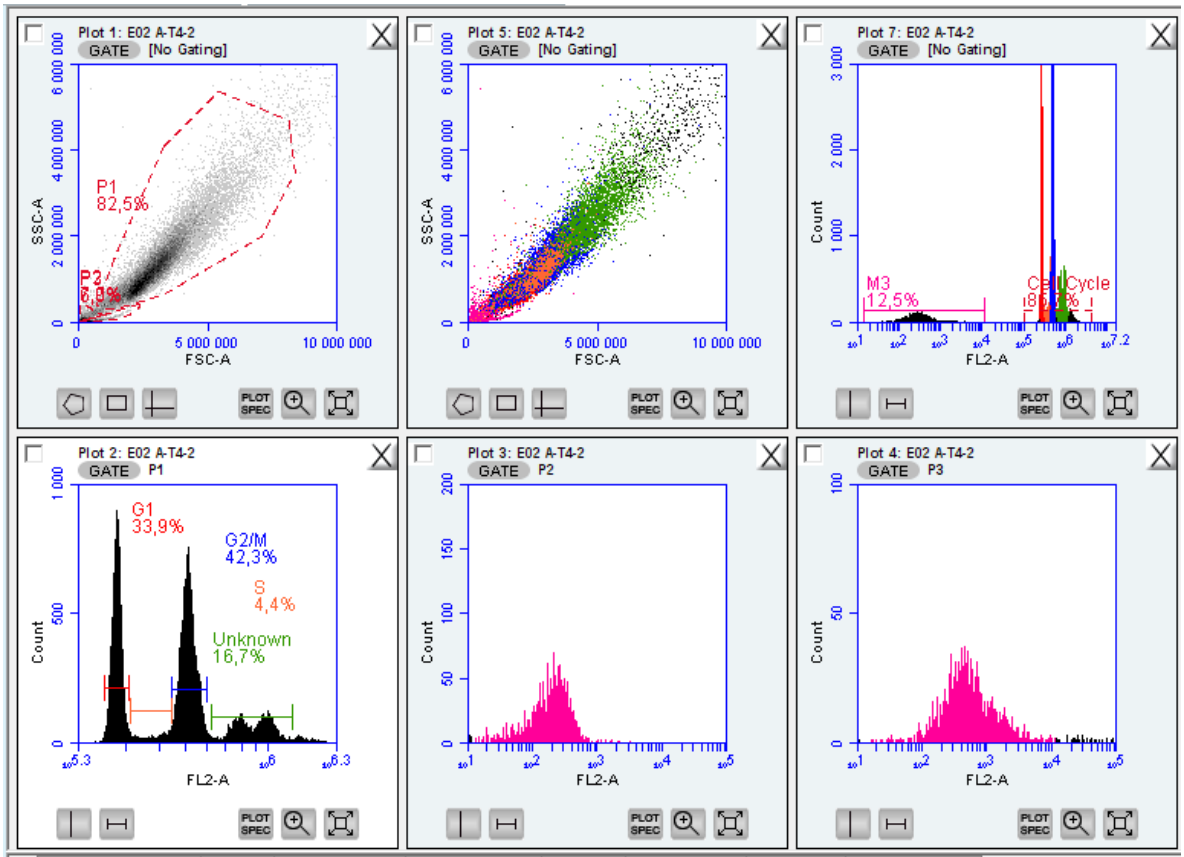
Plot 2: E01 A-T4-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	32 144	155	100,00%	80,36%	557 783,13	51,71%	
G1 (246 553,0 / 30…	8 869	155	27,59%	22,17%	277 835,05	4,00%	
G2/M (438 360,0 / …	13 691	155	42,59%	34,23%	514 976,73	6,32%	
Unknown (615 902,…	7 058	155	21,96%	17,64%	908 775,86	15,44%	
S (307 943,0 / 444 …	1 233	155	3,84%	3,08%	376 484,02	11,35%	

Plot 3: E01 A-T4-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 349	155	100,00%	13,37%	890,94	1 739,32%	

Plot 4: E01 A-T4-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 104	155	100,00%	2,76%	14 499,35	499,34%	

Figure 98. 20 °C at 8 hours sample Nr. 1.

Appendix C23



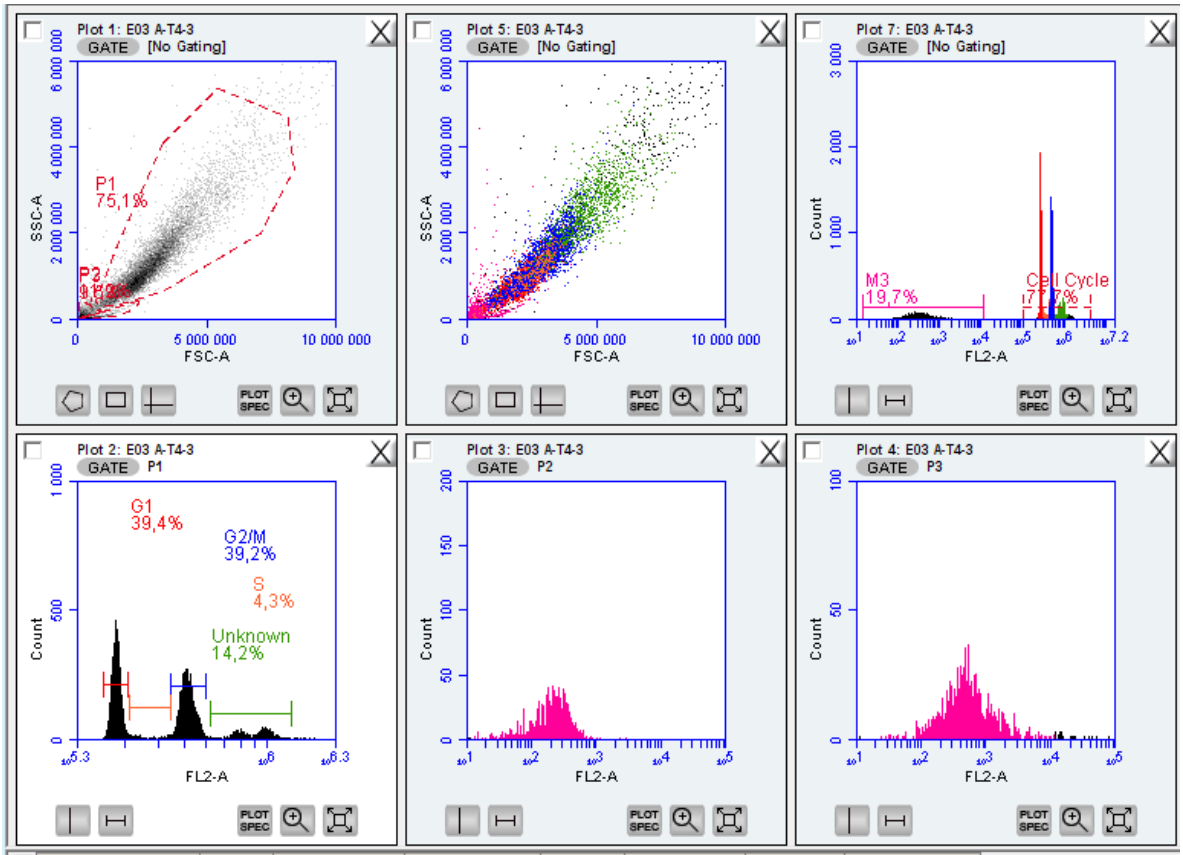
Plot 2: E02 A-T4-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	19 976	233	100,00%	82,45%	504 579,71	51,27%	
G1 (246 553,0 / 30…	6 771	233	33,90%	27,95%	275 917,70	4,00%	
G2/M (438 360,0 / …	8 447	233	42,29%	34,86%	510 278,67	6,32%	
Unknown (615 902,…	3 332	233	16,68%	13,75%	894 605,78	15,55%	
S (307 943,0 / 444 …	883	233	4,42%	3,64%	380 422,30	11,35%	

Plot 3: E02 A-T4-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 906	233	100,00%	7,87%	3 510,05	1 023,80%	

Plot 4: E02 A-T4-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 461	233	100,00%	6,03%	48 404,45	236,33%	

Figure 99. 20 °C at 8 hours sample Nr. 2.

Appendix C24



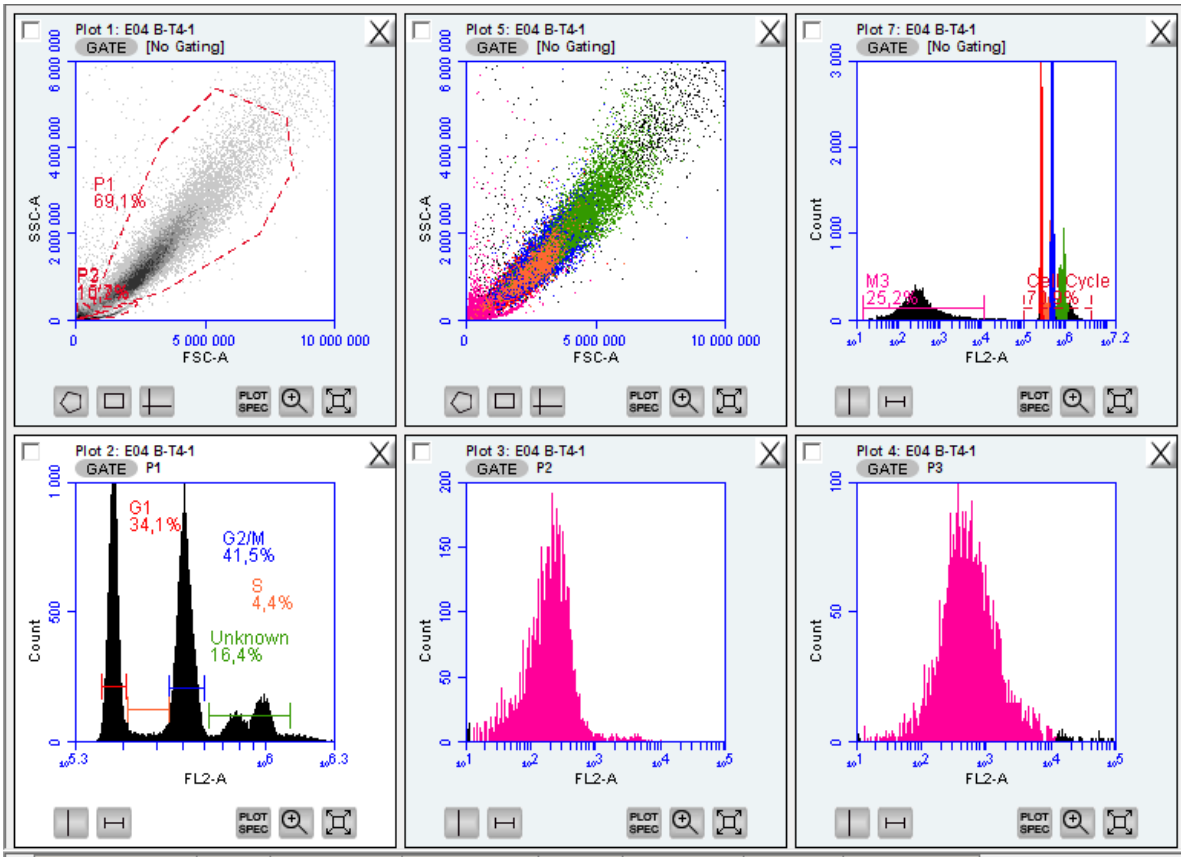
Plot 2: E03 A-T4-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	8 186	199	100,00%	75,09%	478 066,43	52,84%	
G1 (246 553,0 / 30…	3 227	199	39,42%	29,60%	276 986,53	3,76%	
G2/M (438 360,0 / …	3 212	199	39,24%	29,46%	510 999,04	6,04%	
Unknown (615 902,…	1 164	199	14,22%	10,68%	899 547,65	15,53%	
S (307 943,0 / 444 …	348	199	4,25%	3,19%	370 665,70	12,03%	

Plot 3: E03 A-T4-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 222	199	100,00%	11,21%	3 509,69	929,80%	

Plot 4: E03 A-T4-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 032	199	100,00%	9,47%	22 866,03	382,69%	

Figure 100. 20 °C at 8 hours sample Nr. 3.

Appendix C25



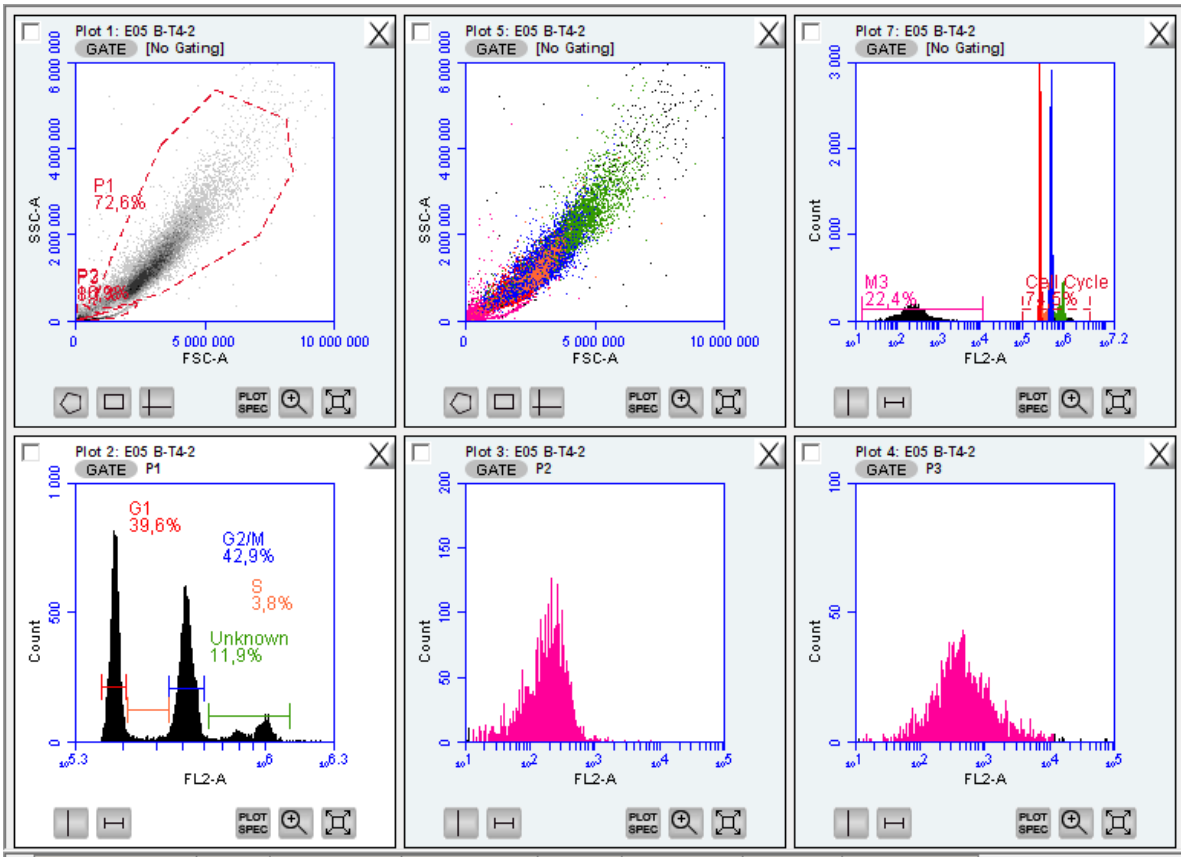
Plot 2: E04 B-T4-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	26 135	344	100,00%	69,09%	495 972,66	52,91%	
G1 (246 553,0 / 30…	8 918	344	34,12%	23,57%	274 299,93	4,07%	
G2/M (438 360,0 / …	10 846	344	41,50%	28,67%	506 784,12	6,24%	
Unknown (615 902…	4 298	344	16,45%	11,36%	901 930,29	14,95%	
S (307 943,0 / 444 …	1 151	344	4,40%	3,04%	378 760,43	11,97%	

Plot 3: E04 B-T4-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 939	344	100,00%	15,70%	1 532,98	1 527,08%	

Plot 4: E04 B-T4-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 867	344	100,00%	10,22%	12 977,38	479,98%	

Figure 101. 26 °C at 8 hours sample Nr. 1.

Appendix C26



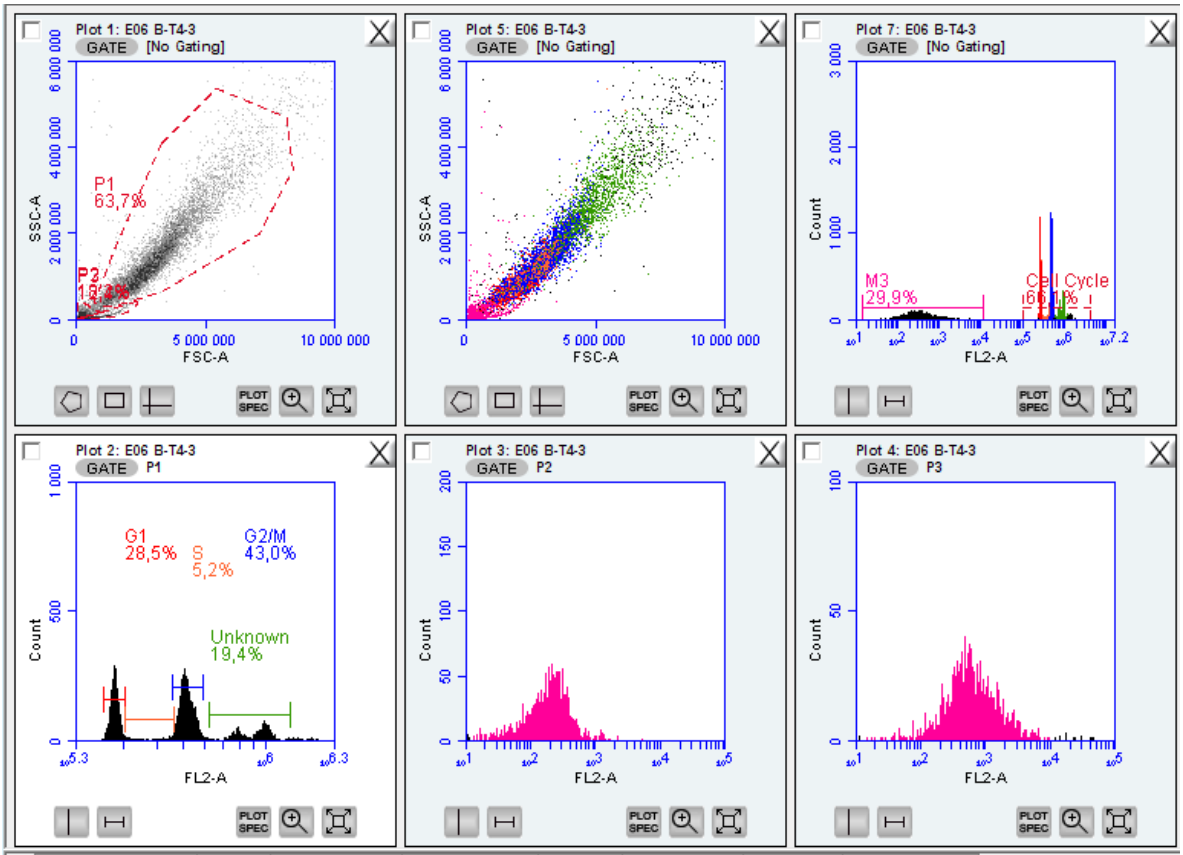
Plot 2: E05 B-T4-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	15 179	196	100,00%	72,59%	468 701,08	49,52%	
G1 (246 553,0 / 30…	6 017	196	39,64%	28,78%	277 888,26	3,89%	
G2/M (438 360,0 / …	6 517	196	42,93%	31,17%	513 742,83	5,93%	
Unknown (615 902…	1 801	196	11,87%	8,61%	916 325,49	14,62%	
S (307 943,0 / 444 …	584	196	3,85%	2,79%	373 181,79	11,82%	

Plot 3: E05 B-T4-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 406	196	100,00%	16,29%	1 329,88	1 559,08%	

Plot 4: E05 B-T4-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 686	196	100,00%	8,06%	22 125,00	360,63%	

Figure 102. 26 °C at 8 hours sample Nr. 2.

Appendix C27



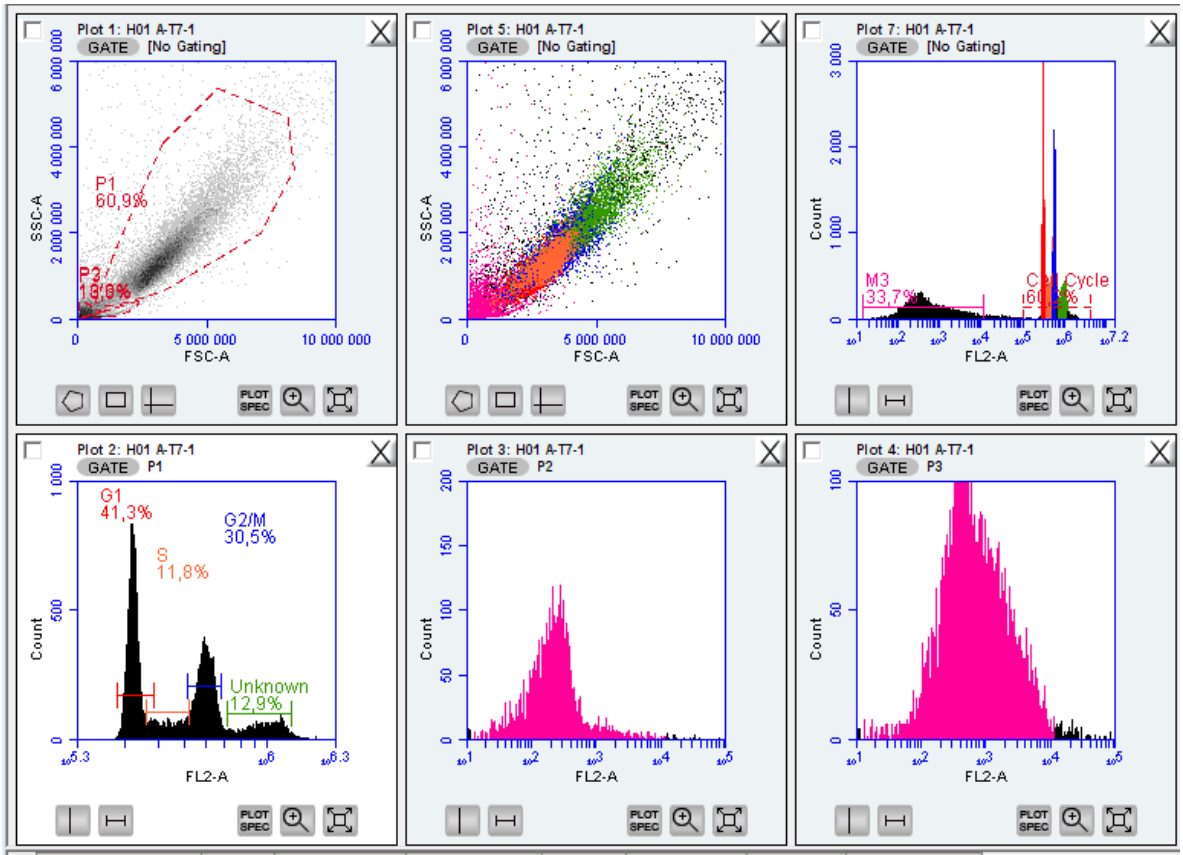
Plot 2: E06 B-T4-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	6 587	205	100,00%	63,73%	533 859,15	51,84%	
G1 (249 799,0 / 30…	1 878	205	28,51%	18,17%	276 364,29	3,53%	
G2/M (449 978,0 / …	2 834	205	43,02%	27,42%	512 675,05	5,62%	
Unknown (615 902…	1 281	205	19,45%	12,39%	915 138,51	14,53%	
S (299 993,0 / 461 …	342	205	5,19%	3,31%	385 940,48	14,20%	

Plot 3: E06 B-T4-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 882	205	100,00%	18,21%	857,01	1 410,11%	

Plot 4: E06 B-T4-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 286	205	100,00%	12,44%	9 510,57	519,47%	

Figure 103. 26 °C at 8 hours sample Nr. 3.

Appendix C28



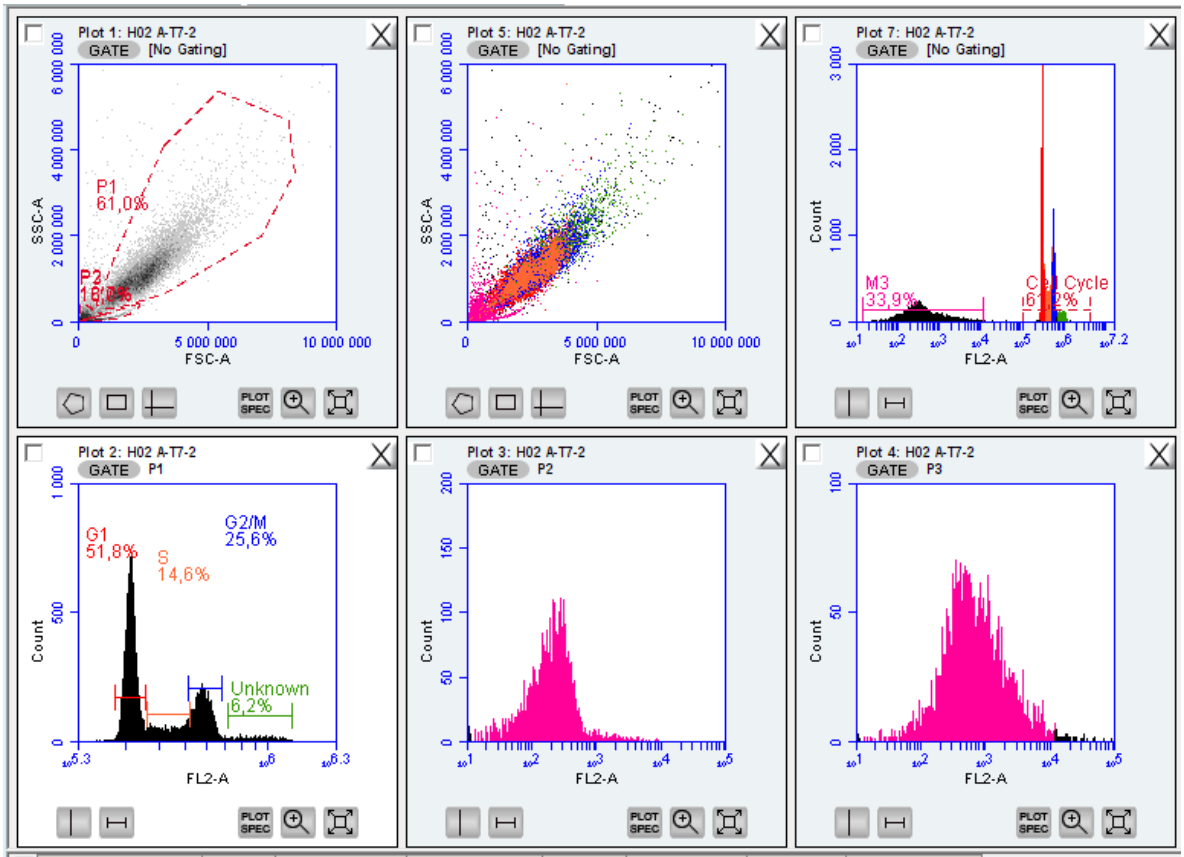
Plot 2: H01 A-T7-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	18 269	232	100,00%	60,90%	504 762,04	54,29%	
G1 (277 351,0 / 38...	7 536	232	41,25%	25,12%	322 124,63	5,76%	
G2/M (506 187,0 / ...	5 564	232	30,46%	18,55%	593 639,08	6,90%	
Unknown (711 200,...	2 360	232	12,92%	7,87%	974 612,19	14,56%	
S (355 591,0 / 519 ...	2 147	232	11,75%	7,16%	433 922,55	11,45%	

Plot 3: H01 A-T7-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	4 093	232	100,00%	13,64%	1 403,39	1 008,25%	

Plot 4: H01 A-T7-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 501	232	100,00%	18,34%	2 992,10	732,75%	

Figure 104. 20 °C at 26 hours sample Nr. 1.

Appendix C29



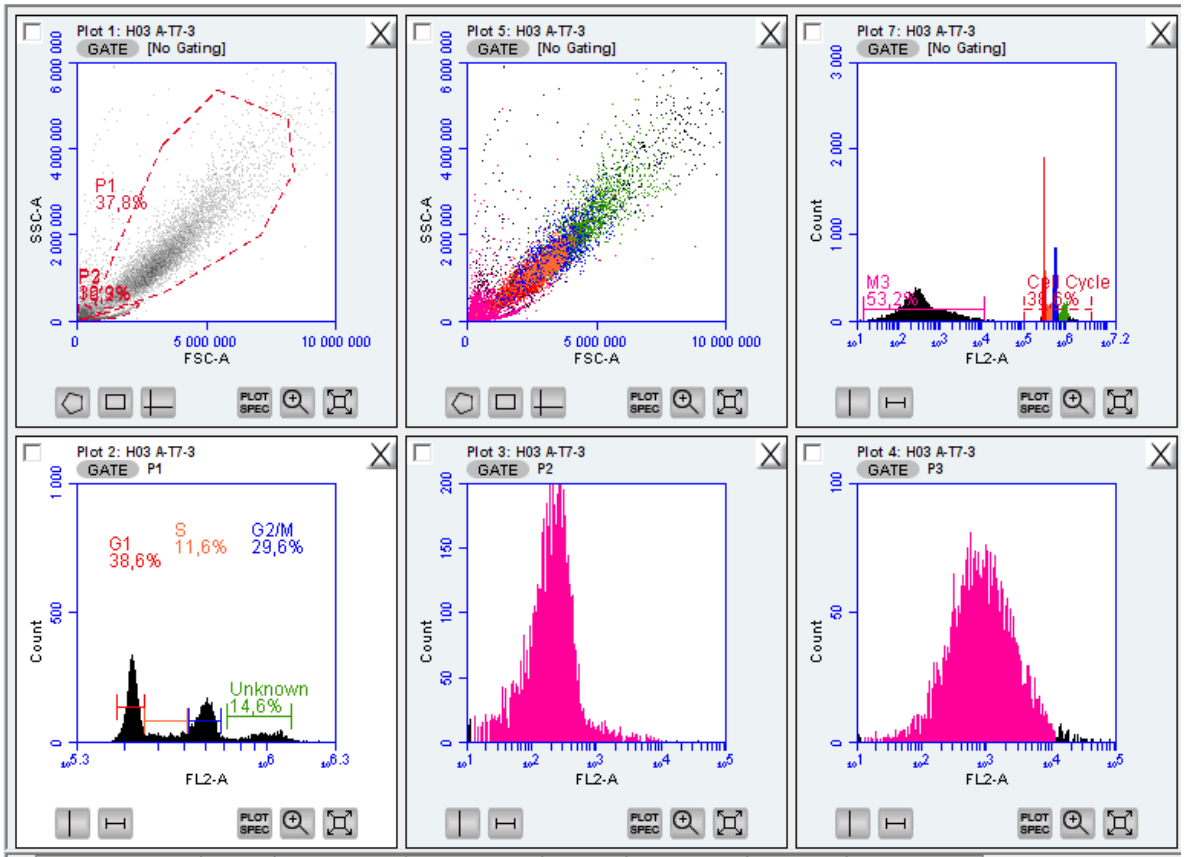
Plot 2: H02 A-T7-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	12 190	271	100,00%	60,95%	436 423,82	45,85%	
G1 (270 191,0 / 35...	6 317	271	51,82%	31,58%	311 555,45	4,80%	
G2/M (506 187,0 / ...	3 123	271	25,62%	15,62%	578 931,70	6,80%	
Unknown (711 200,...	753	271	6,18%	3,76%	937 503,09	14,72%	
S (355 591,0 / 519 ...	1 785	271	14,64%	8,92%	437 067,67	11,55%	

Plot 3: H02 A-T7-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 734	271	100,00%	18,67%	1 110,82	1 984,15%	

Plot 4: H02 A-T7-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 200	271	100,00%	16,00%	7 406,62	611,03%	

Figure 105. 20 °C at 26 hours sample Nr. 2.

Appendix C30



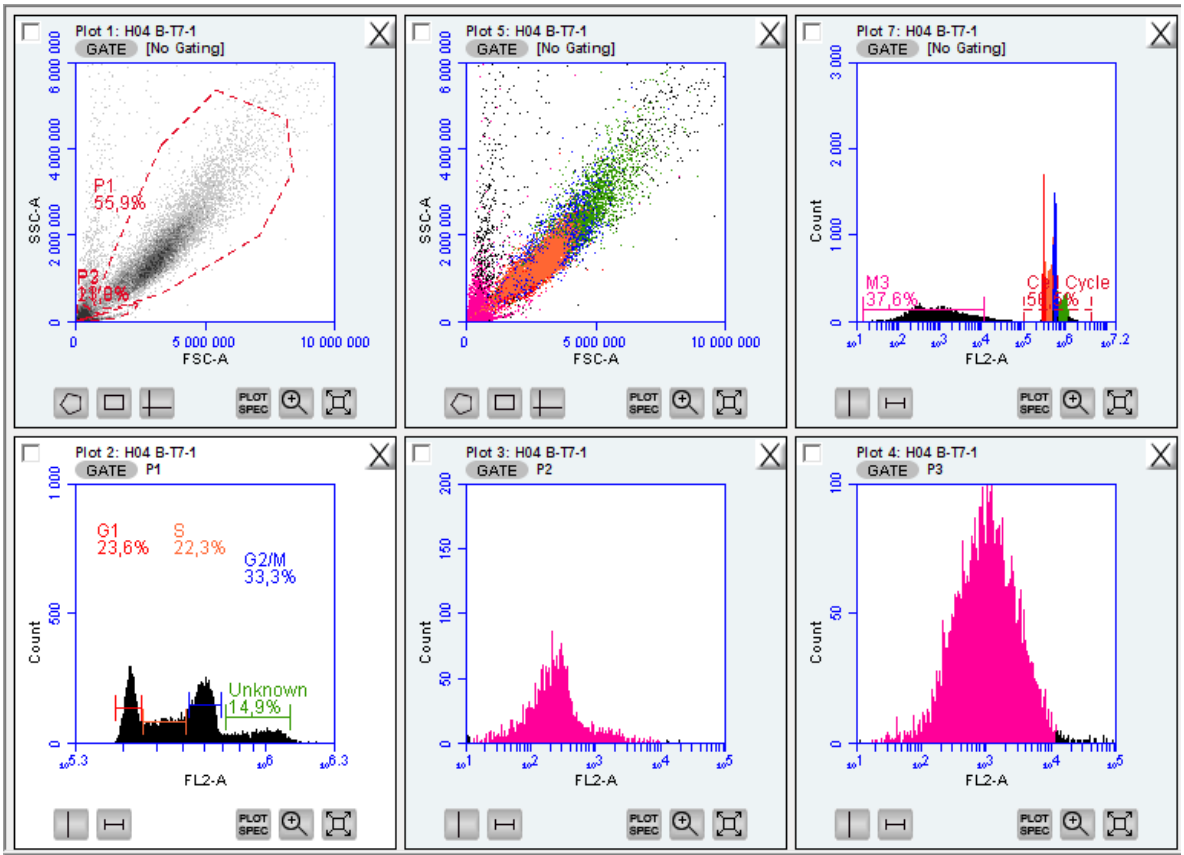
Plot 2: H03 A-T7-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	7 566	386	100,00%	37,83%	525 233,44	52,69%	
G1 (277 351,0 / 35...	2 921	386	38,61%	14,60%	318 407,24	4,70%	
G2/M (512 851,0 / ...	2 243	386	29,65%	11,22%	594 228,20	6,57%	
Unknown (711 200,...	1 107	386	14,63%	5,54%	978 335,79	13,92%	
S (350 971,0 / 512 ...	877	386	11,59%	4,38%	429 471,32	11,43%	

Plot 3: H03 A-T7-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	7 388	386	100,00%	36,94%	568,67	1 971,49%	

Plot 4: H03 A-T7-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 845	386	100,00%	19,22%	3 990,07	721,83%	

Figure 106. 20 °C at 26 hours sample Nr. 3.

Appendix C31



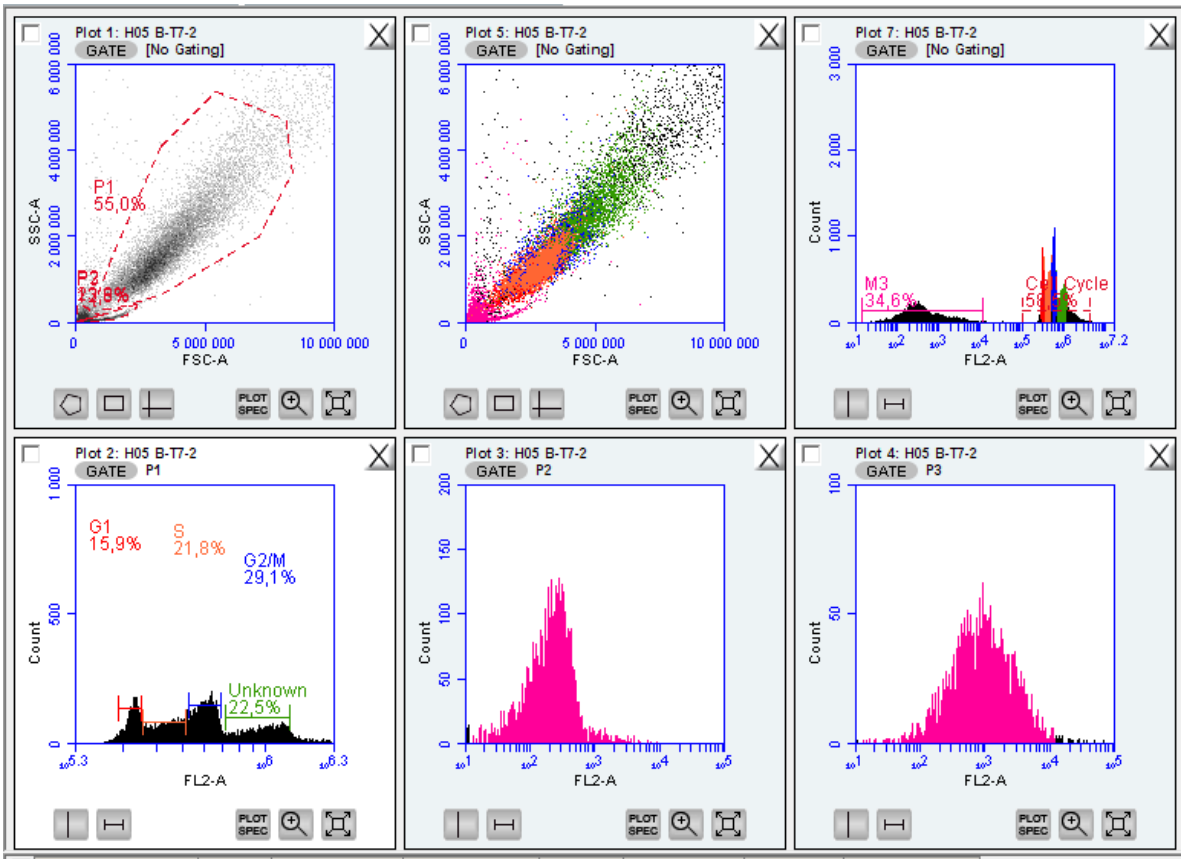
Plot 2: H04 B-T7-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	11 756	196	100,00%	55,91%	545 995,00	47,23%	
G1 (277 351,0 / 35...	2 775	196	23,60%	13,20%	317 663,08	4,75%	
G2/M (519 602,0 / ...	3 919	196	33,34%	18,64%	593 190,48	6,97%	
Unknown (711 200,...	1 756	196	14,94%	8,35%	958 065,89	14,94%	
S (350 971,0 / 512 ...	2 622	196	22,30%	12,47%	431 859,40	10,91%	

Plot 3: H04 B-T7-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 726	196	100,00%	12,96%	936,53	1 334,62%	

Plot 4: H04 B-T7-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	4 591	196	100,00%	21,83%	3 741,82	760,41%	

Figure 107. 26 °C at 26 hours sample Nr. 1.

Appendix C32



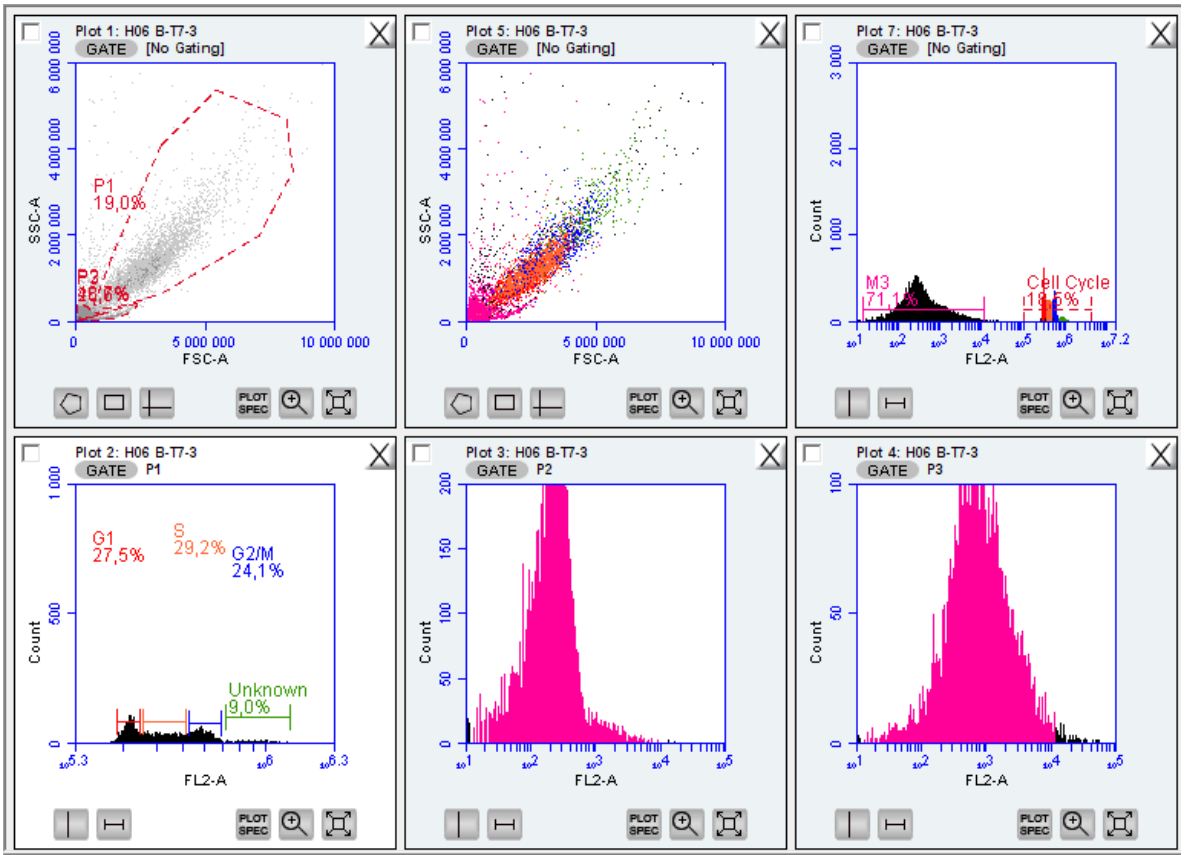
Plot 2: H05 B-T7-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	10 996	327	100,00%	54,98%	639 908,11	51,94%	
G1 (284 702,0 / 35...	1 746	327	15,88%	8,73%	323 121,14	5,01%	
G2/M (519 602,0 / ...	3 202	327	29,12%	16,01%	603 510,39	7,59%	
Unknown (711 200,...	2 476	327	22,52%	12,38%	980 429,24	15,18%	
S (350 971,0 / 512 ...	2 392	327	21,75%	11,96%	432 995,34	10,87%	

Plot 3: H05 B-T7-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	4 753	327	100,00%	23,76%	321,01	618,71%	

Plot 4: H05 B-T7-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 564	327	100,00%	12,82%	3 749,61	813,76%	

Figure 108. 26 °C at 26 hours sample Nr. 2.

Appendix C33



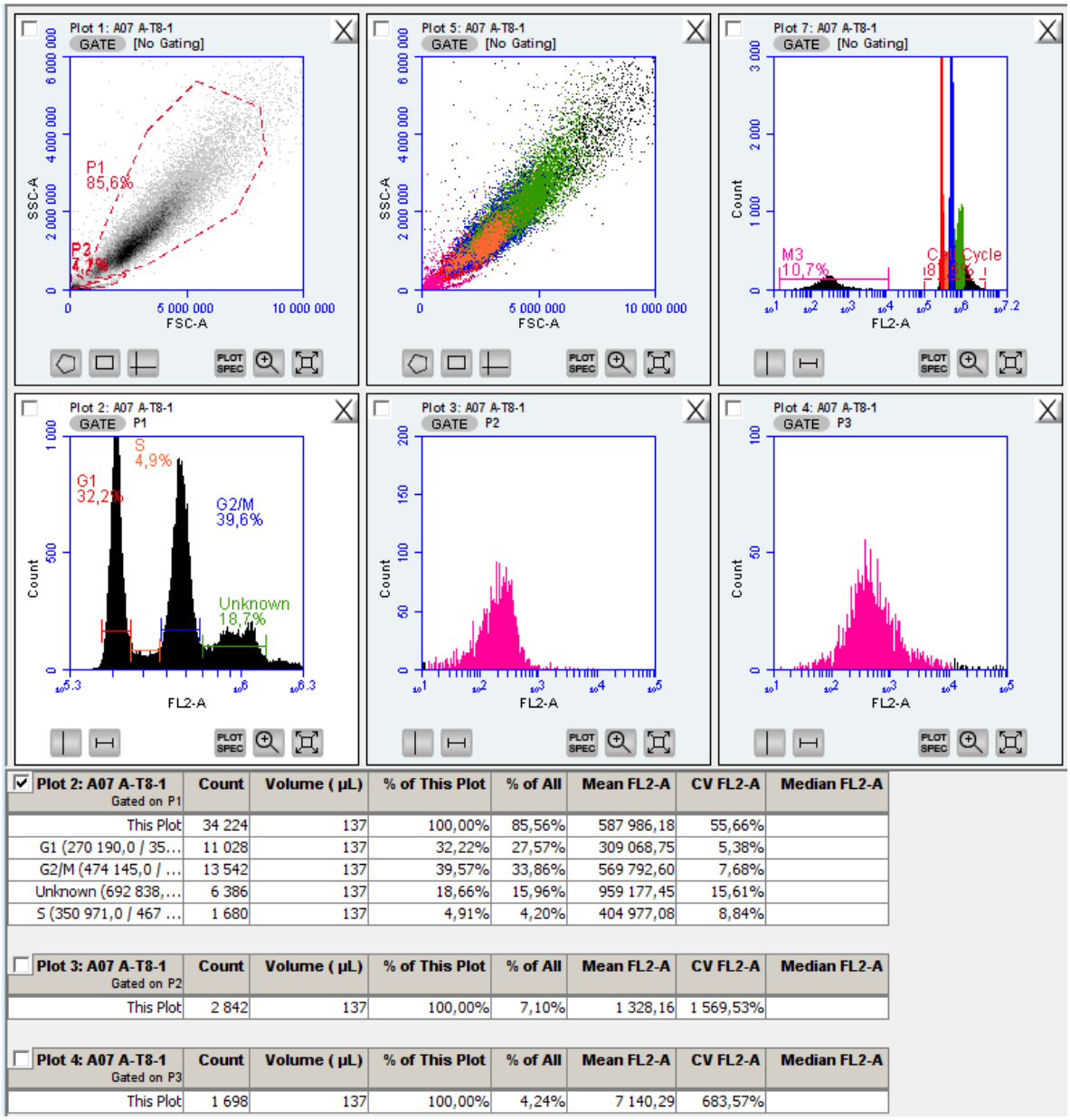
Plot 2: H06 B-T7-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 800	300	100,00%	19,00%	462 954,40	49,26%	
G1 (281 003,0 / 34...	1 046	300	27,53%	5,23%	314 955,71	4,88%	
G2/M (519 602,0 / ...	914	300	24,05%	4,57%	589 908,10	7,22%	
Unknown (711 200,...	341	300	8,97%	1,70%	924 843,59	14,60%	
S (350 971,0 / 512 ...	1 111	300	29,24%	5,56%	426 454,43	10,95%	

Plot 3: H06 B-T7-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	9 733	300	100,00%	48,67%	423,81	1 690,05%	

Plot 4: H06 B-T7-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 317	300	100,00%	26,58%	2 424,21	868,99%	

Figure 109. 26 °C at 26 hours sample Nr. 3.

Appendix C34



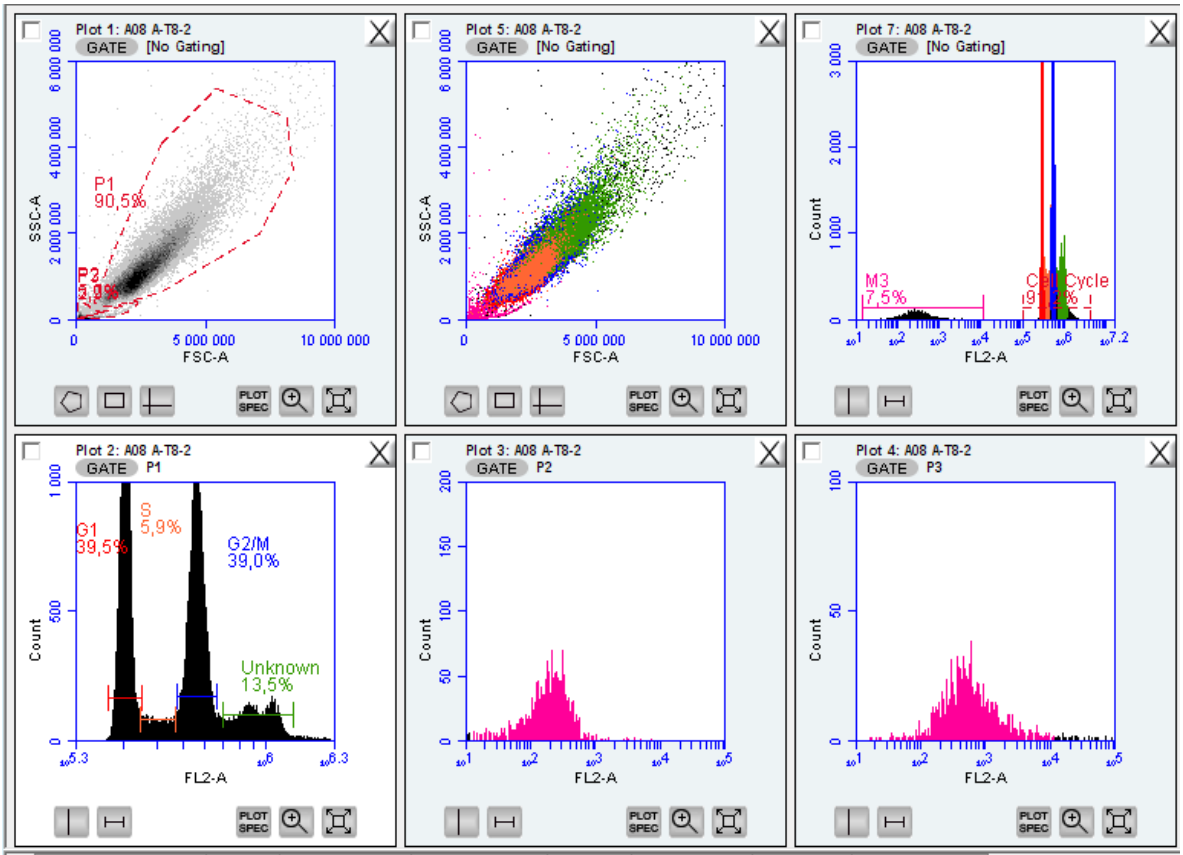
Plot 2: A07 A-T8-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	34 224	137	100,00%	85,56%	587 986,18	55,66%	
G1 (270 190,0 / 35...	11 028	137	32,22%	27,57%	309 068,75	5,38%	
G2/M (474 145,0 / ...	13 542	137	39,57%	33,86%	569 792,60	7,68%	
Unknown (692 838,...	6 386	137	18,66%	15,96%	959 177,45	15,61%	
S (350 971,0 / 467 ...	1 680	137	4,91%	4,20%	404 977,08	8,84%	

Plot 3: A07 A-T8-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 842	137	100,00%	7,10%	1 328,16	1 569,53%	

Plot 4: A07 A-T8-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 698	137	100,00%	4,24%	7 140,29	683,57%	

Figure 110. 20 °C at 30 hours sample Nr. 1.

Appendix C35



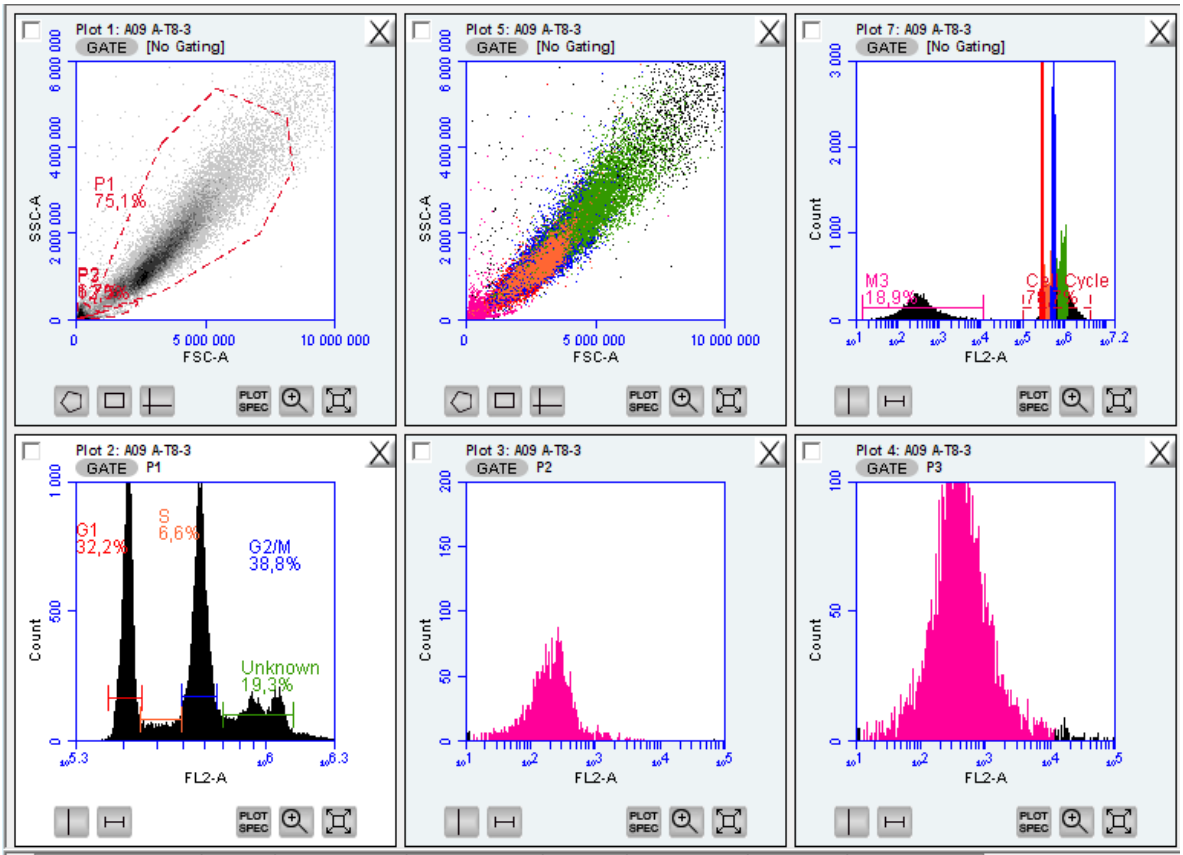
Plot 2: A08 A-T8-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	36 204	167	100,00%	90,51%	510 317,52	47,95%	
G1 (259 795,0 / 350…	14 304	167	39,51%	35,76%	303 362,85	4,69%	
G2/M (474 145,0 / 6…	14 115	167	38,99%	35,29%	559 361,33	6,75%	
Unknown (692 838,…	4 870	167	13,45%	12,17%	935 042,72	15,04%	
S (341 909,0 / 467 9…	2 128	167	5,88%	5,32%	401 082,59	9,33%	

Plot 3: A08 A-T8-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 068	167	100,00%	5,17%	973,56	1 412,25%	

Plot 4: A08 A-T8-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 146	167	100,00%	2,86%	13 862,06	504,88%	

Figure 111. 20 °C at 30 hours sample Nr. 2.

Appendix C36



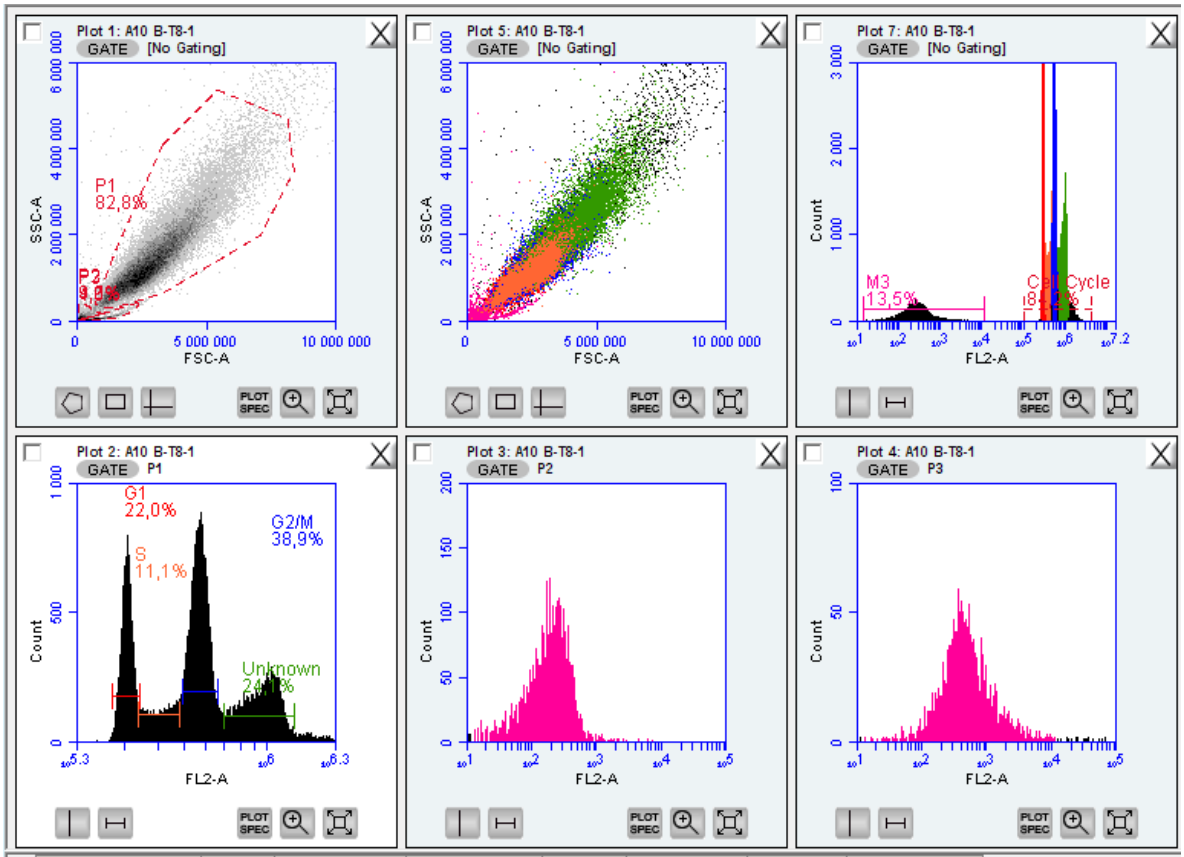
Plot 2: A09 A-T8-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	30 046	308	100,00%	75,12%	570 785,54	49,67%	
G1 (259 795,0 / 35...	9 667	308	32,17%	24,17%	308 271,53	4,77%	
G2/M (486 711,0 / ...	11 672	308	38,85%	29,18%	573 837,67	6,42%	
Unknown (692 838,...	5 789	308	19,27%	14,47%	961 533,73	15,64%	
S (341 909,0 / 493 ...	1 994	308	6,64%	4,98%	416 423,52	11,22%	

Plot 3: A09 A-T8-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 698	308	100,00%	6,74%	3 169,33	1 248,05%	

Plot 4: A09 A-T8-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 008	308	100,00%	12,52%	5 835,45	733,36%	

Figure 112. 20 °C at 30 hours sample Nr. 3.

Appendix C37



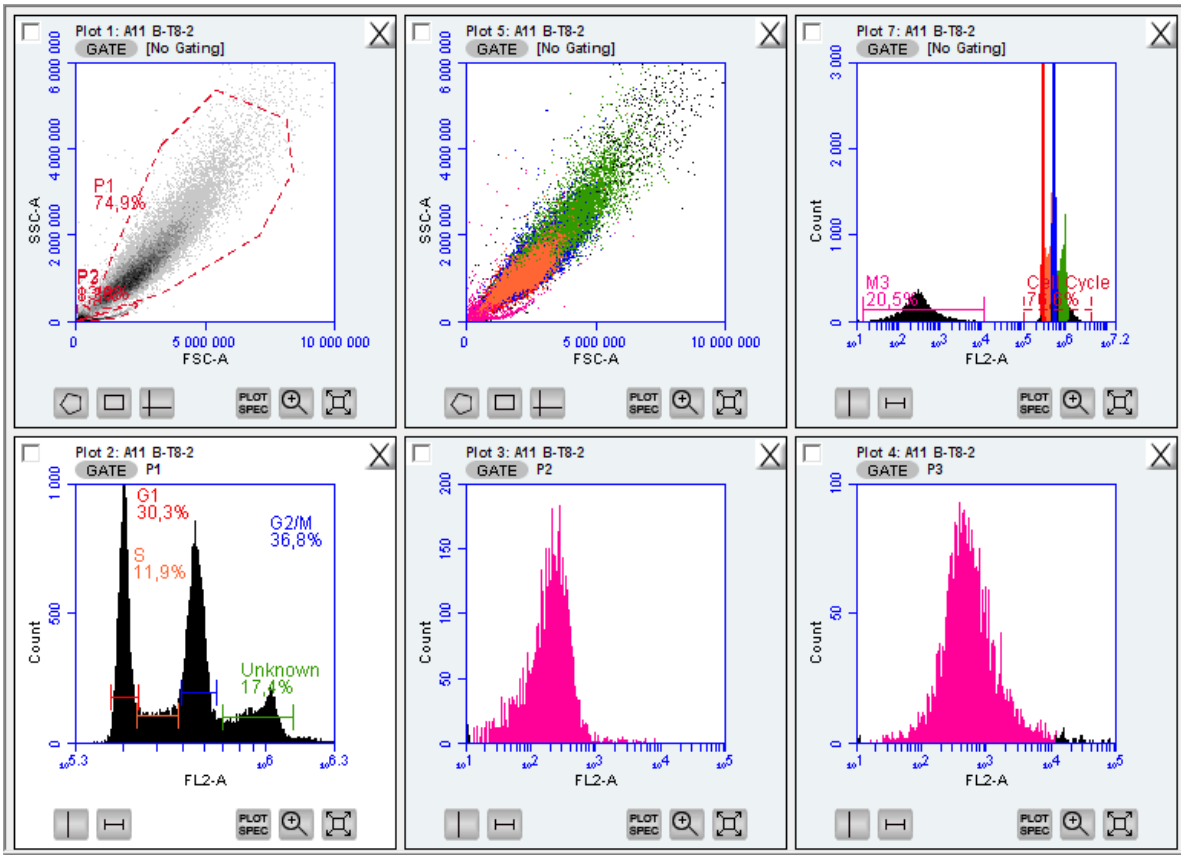
Plot 2: A10 B-T8-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	33 132	137	100,00%	82,83%	608 921,11	48,54%	
G1 (266 680,0 / 34…	7 273	137	21,95%	18,18%	305 083,20	4,93%	
G2/M (486 711,0 / …	12 881	137	38,88%	32,20%	567 284,82	7,13%	
Unknown (692 838,…	7 987	137	24,11%	19,97%	955 581,75	15,02%	
S (333 082,0 / 480 …	3 683	137	11,12%	9,21%	404 828,58	10,95%	

Plot 3: A10 B-T8-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 965	137	100,00%	9,91%	1 349,42	1 709,76%	

Plot 4: A10 B-T8-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 855	137	100,00%	4,64%	8 477,39	589,85%	

Figure 113. 26 °C at 30 hours sample Nr. 1.

Appendix C38



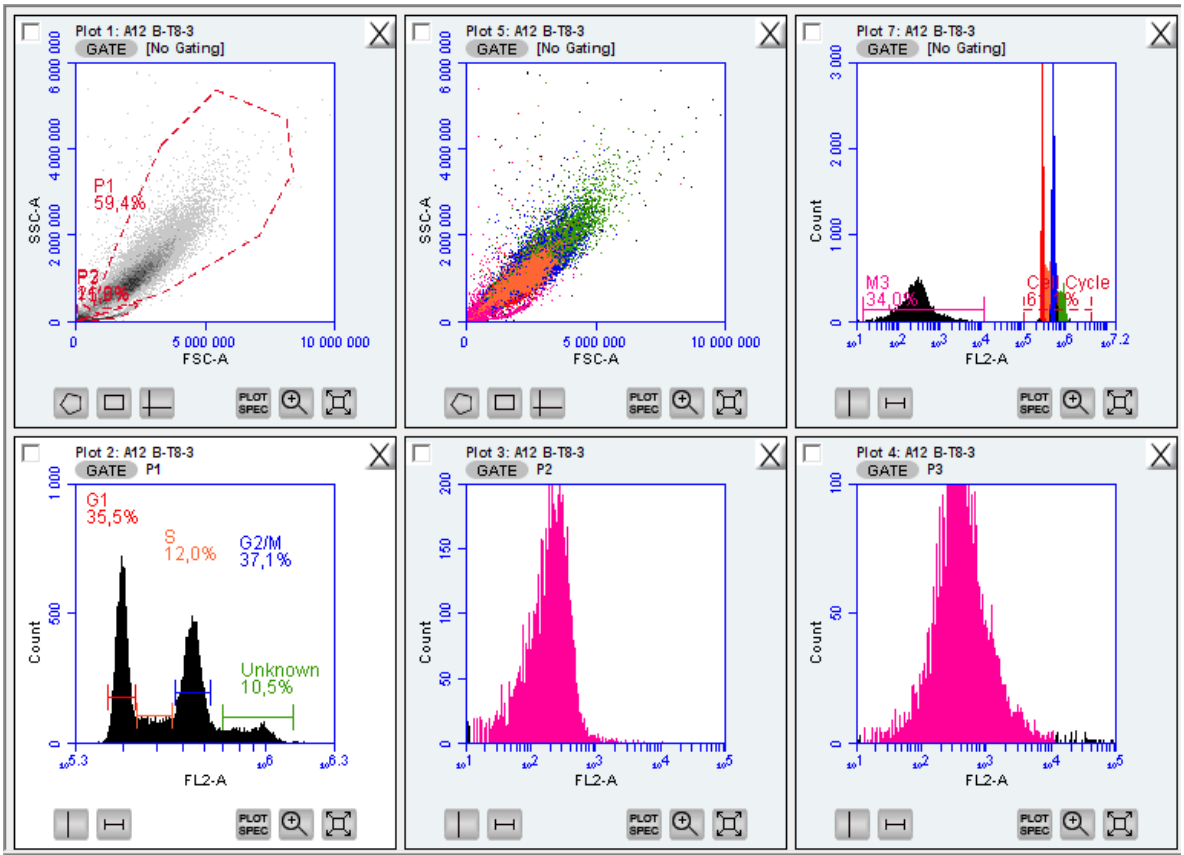
Plot 2: A11 B-T8-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	29 947	297	100,00%	74,87%	546 757,18	50,59%	
G1 (266 680,0 / 34...	9 059	297	30,25%	22,65%	301 090,17	4,83%	
G2/M (486 711,0 / ...	11 029	297	36,83%	27,57%	559 480,96	6,88%	
Unknown (692 838,...	5 211	297	17,40%	13,03%	942 109,40	14,64%	
S (333 082,0 / 480 ...	3 555	297	11,87%	8,89%	404 647,03	10,88%	

Plot 3: A11 B-T8-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 502	297	100,00%	13,76%	515,24	1 670,19%	

Plot 4: A11 B-T8-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 339	297	100,00%	8,35%	14 728,11	516,69%	

Figure 114. 26 °C at 30 hours sample Nr. 2.

Appendix C39



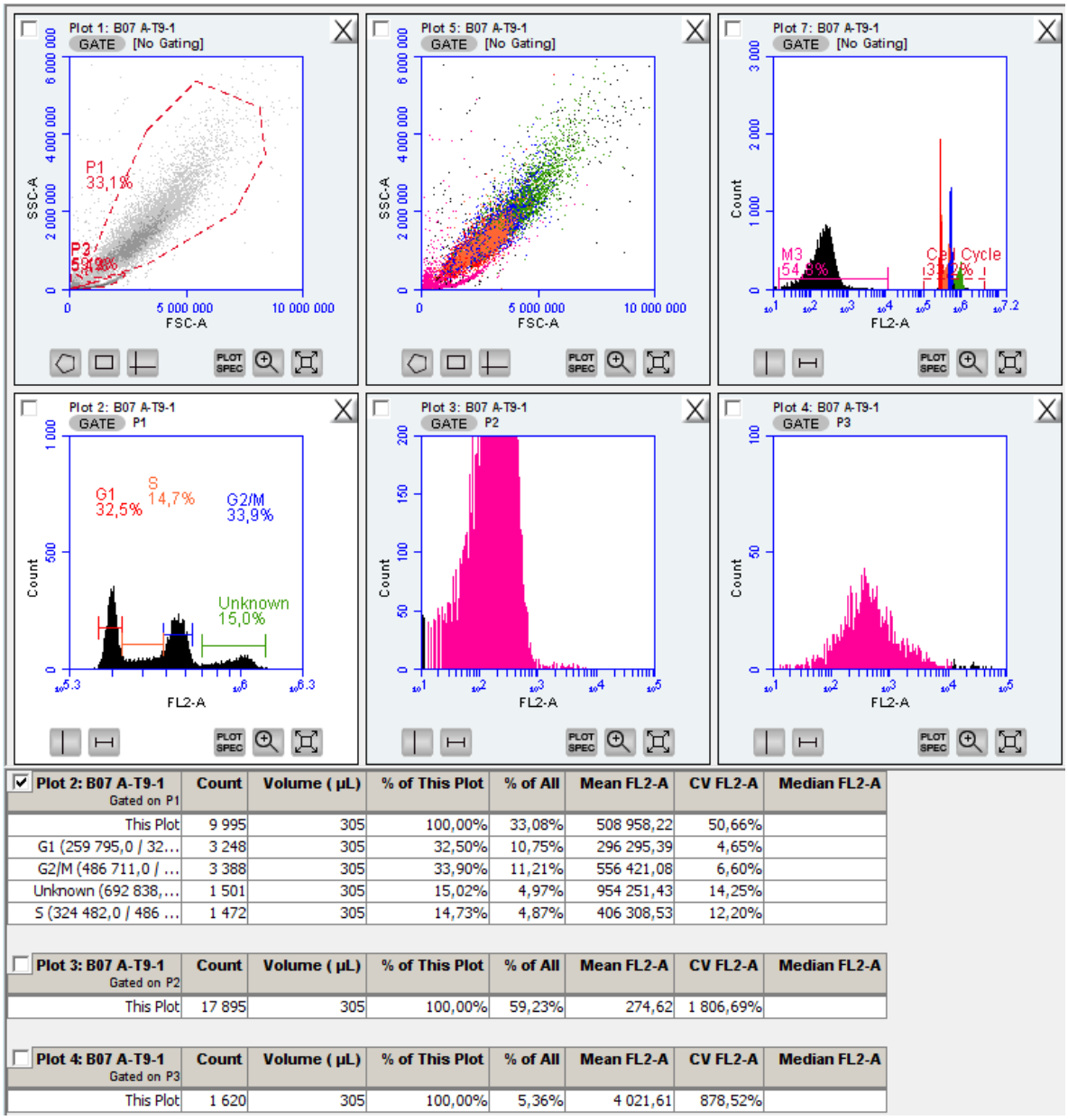
Plot 2: A12 B-T8-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	19 280	393	100,00%	59,42%	473 849,32	45,52%	
G1 (259 795,0 / 33...	6 843	393	35,49%	21,09%	295 524,55	4,92%	
G2/M (461 903,0 / ...	7 161	393	37,14%	22,07%	539 337,65	7,11%	
Unknown (692 838,...	2 033	393	10,54%	6,27%	895 471,75	14,21%	
S (333 082,0 / 455 ...	2 305	393	11,96%	7,10%	391 519,57	9,27%	

Plot 3: A12 B-T8-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	7 093	393	100,00%	21,86%	1 042,88	2 235,39%	

Plot 4: A12 B-T8-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	4 875	393	100,00%	15,02%	13 965,01	656,16%	

Figure 115. 26 °C at 30 hours sample Nr. 3.

Appendix C40



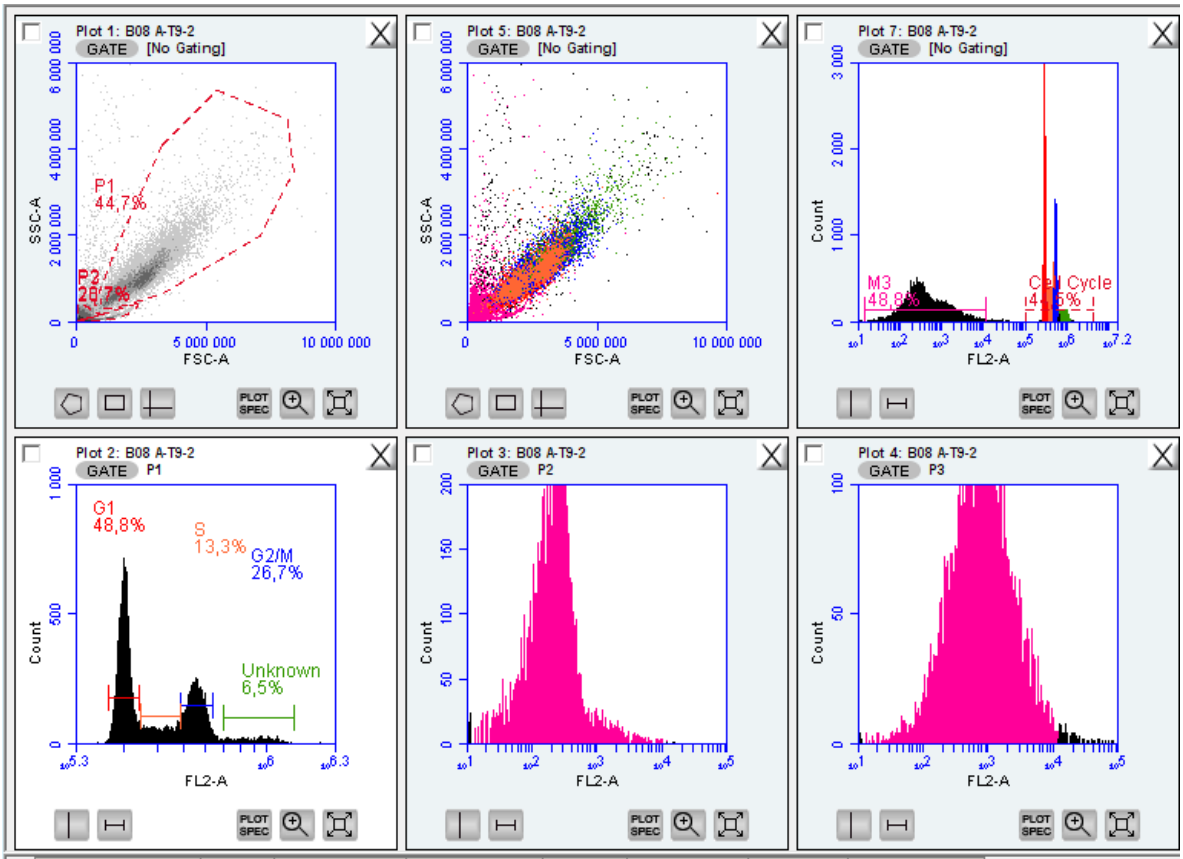
Plot 2: B07 A-T9-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	9 995	305	100,00%	33,08%	508 958,22	50,66%	
G1 (259 795,0 / 32...	3 248	305	32,50%	10,75%	296 295,39	4,65%	
G2/M (486 711,0 / ...	3 388	305	33,90%	11,21%	556 421,08	6,60%	
Unknown (692 838,...	1 501	305	15,02%	4,97%	954 251,43	14,25%	
S (324 482,0 / 486 ...	1 472	305	14,73%	4,87%	406 308,53	12,20%	

Plot 3: B07 A-T9-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	17 895	305	100,00%	59,23%	274,62	1 806,69%	

Plot 4: B07 A-T9-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 620	305	100,00%	5,36%	4 021,61	878,52%	

Figure 116. 20 °C at 42 hours sample Nr. 1.

Appendix C41



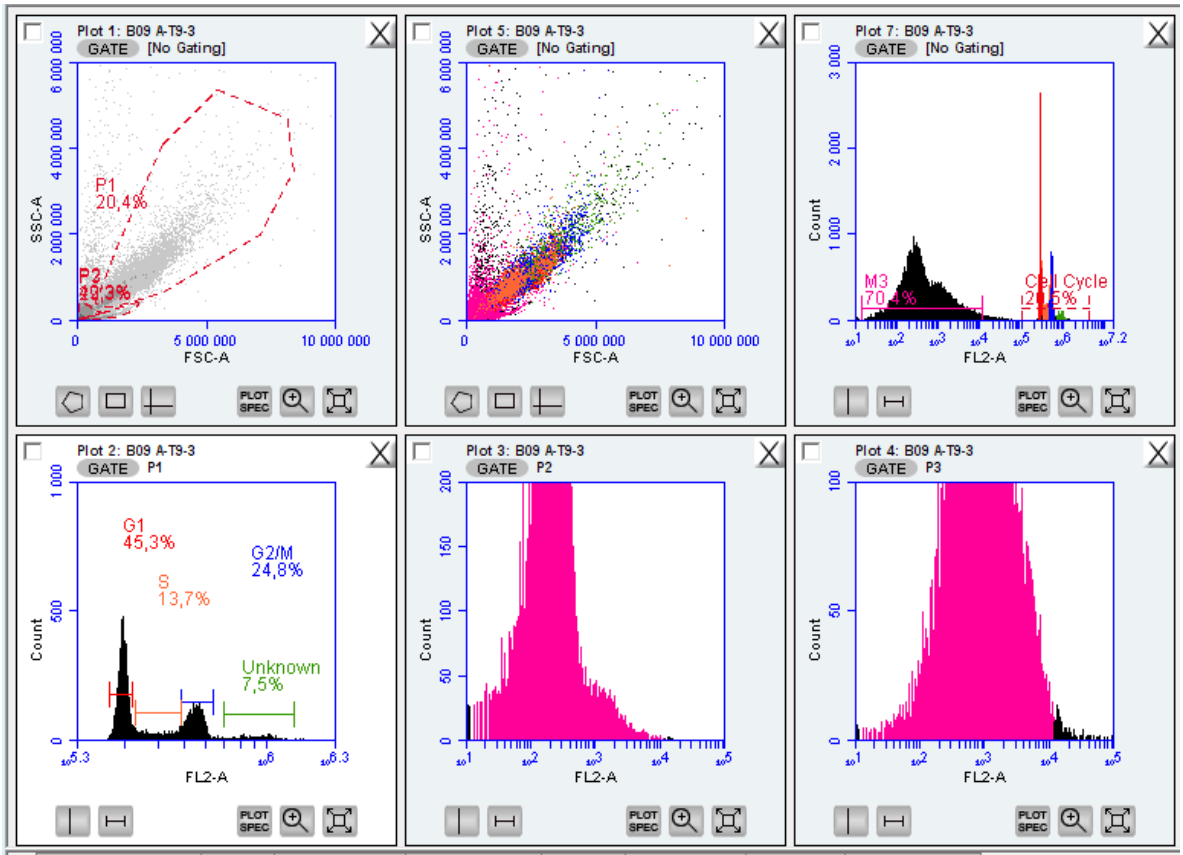
Plot 2: B08 A-T9-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	13 657	295	100,00%	44,65%	426 852,58	48,15%	
G1 (259 795,0 / 34…	6 658	295	48,75%	21,77%	299 342,52	5,15%	
G2/M (486 711,0 / …	3 645	295	26,69%	11,92%	554 700,55	6,64%	
Unknown (692 838,…	892	295	6,53%	2,92%	923 696,60	15,63%	
S (341 909,0 / 486 …	1 821	295	13,33%	5,95%	412 569,24	10,62%	

Plot 3: B08 A-T9-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	8 769	295	100,00%	28,67%	549,91	1 687,36%	

Plot 4: B08 A-T9-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	6 337	295	100,00%	20,72%	3 853,17	707,78%	

Figure 117. 20 °C at 42 hours sample Nr. 2.

Appendix C42



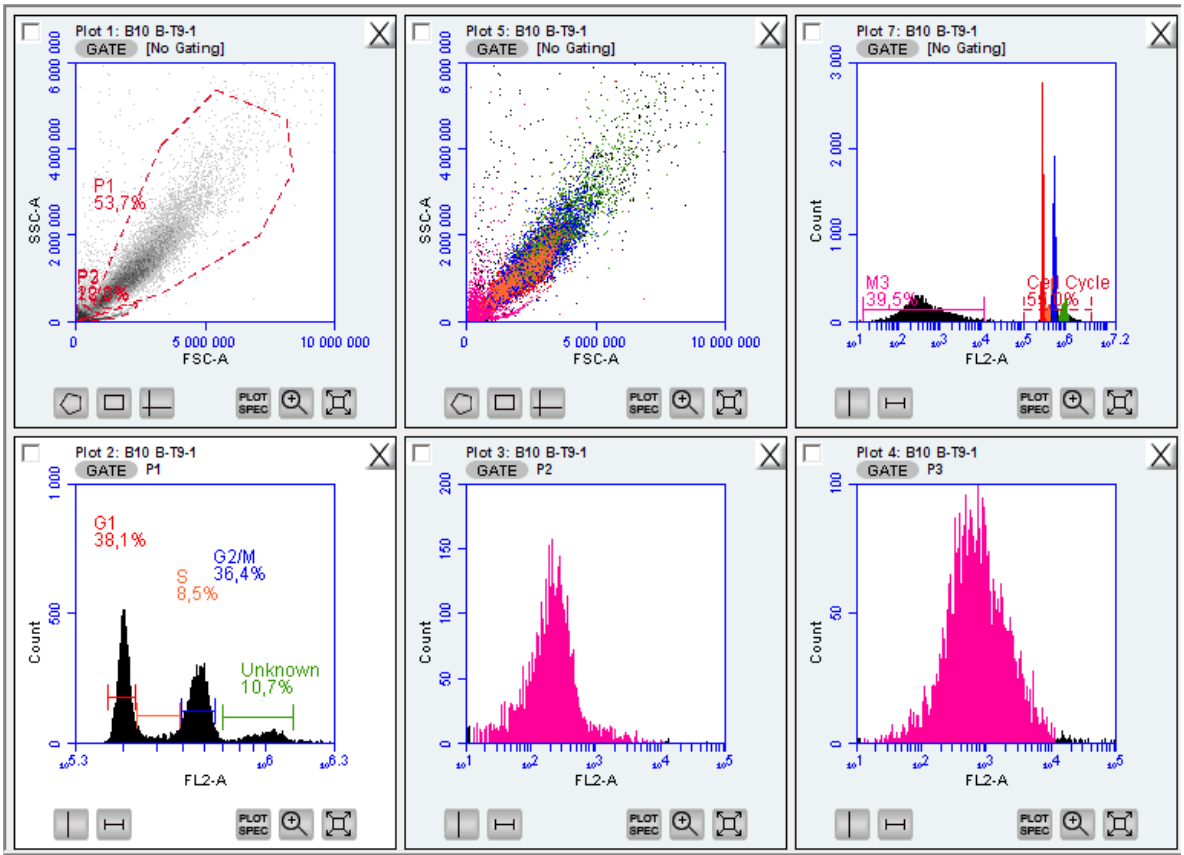
Plot 2: B09 A-T9-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	7 683	287	100,00%	20,36%	423 000,23	58,53%	
G1 (259 795,0 / 32...	3 479	287	45,28%	9,22%	292 918,45	3,90%	
G2/M (486 711,0 / ...	1 908	287	24,83%	5,06%	549 218,94	6,07%	
Unknown (692 838,...	574	287	7,47%	1,52%	932 467,35	14,80%	
S (324 482,0 / 486 ...	1 049	287	13,65%	2,78%	401 449,53	12,62%	

Plot 3: B09 A-T9-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	15 952	287	100,00%	42,28%	427,49	1 592,51%	

Plot 4: B09 A-T9-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	11 071	287	100,00%	29,34%	4 069,02	683,47%	

Figure 118. 20 °C at 42 hours sample Nr. 3.

Appendix C43



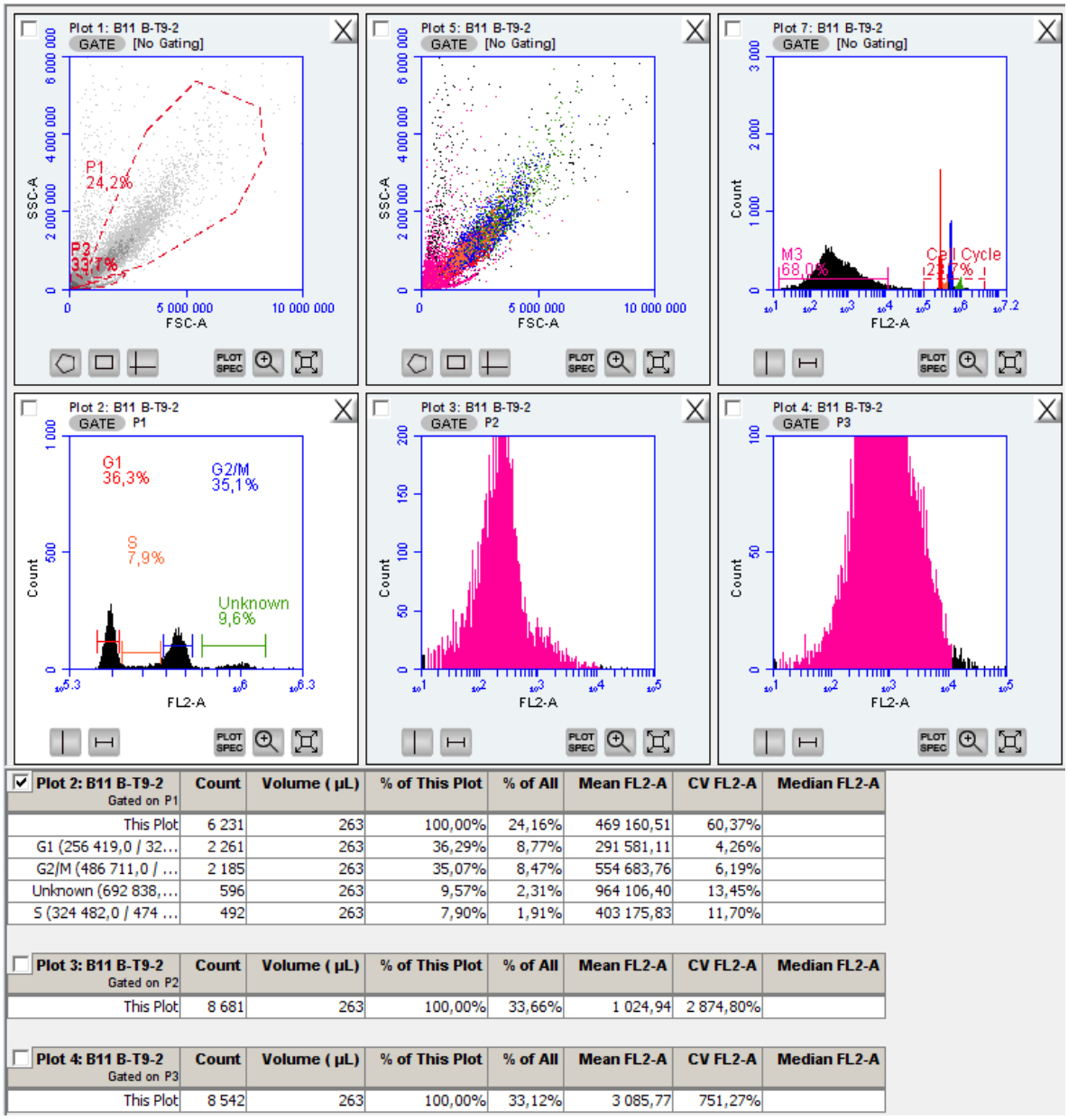
Plot 2: B10 B-T9-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	12 431	235	100,00%	53,67%	509 520,87	56,83%	
G1 (259 795,0 / 33...	4 741	235	38,14%	20,47%	299 457,77	4,72%	
G2/M (493 118,0 / ...	4 522	235	36,38%	19,52%	569 810,88	6,77%	
Unknown (692 838,...	1 331	235	10,71%	5,75%	989 343,81	14,09%	
S (333 081,0 / 486 ...	1 052	235	8,46%	4,54%	406 147,62	12,60%	

Plot 3: B10 B-T9-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 088	235	100,00%	21,97%	638,98	1 604,77%	

Plot 4: B10 B-T9-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	4 170	235	100,00%	18,00%	4 924,96	657,36%	

Figure 119. 26 °C at 42 hours sample Nr. 1.

Appendix C44



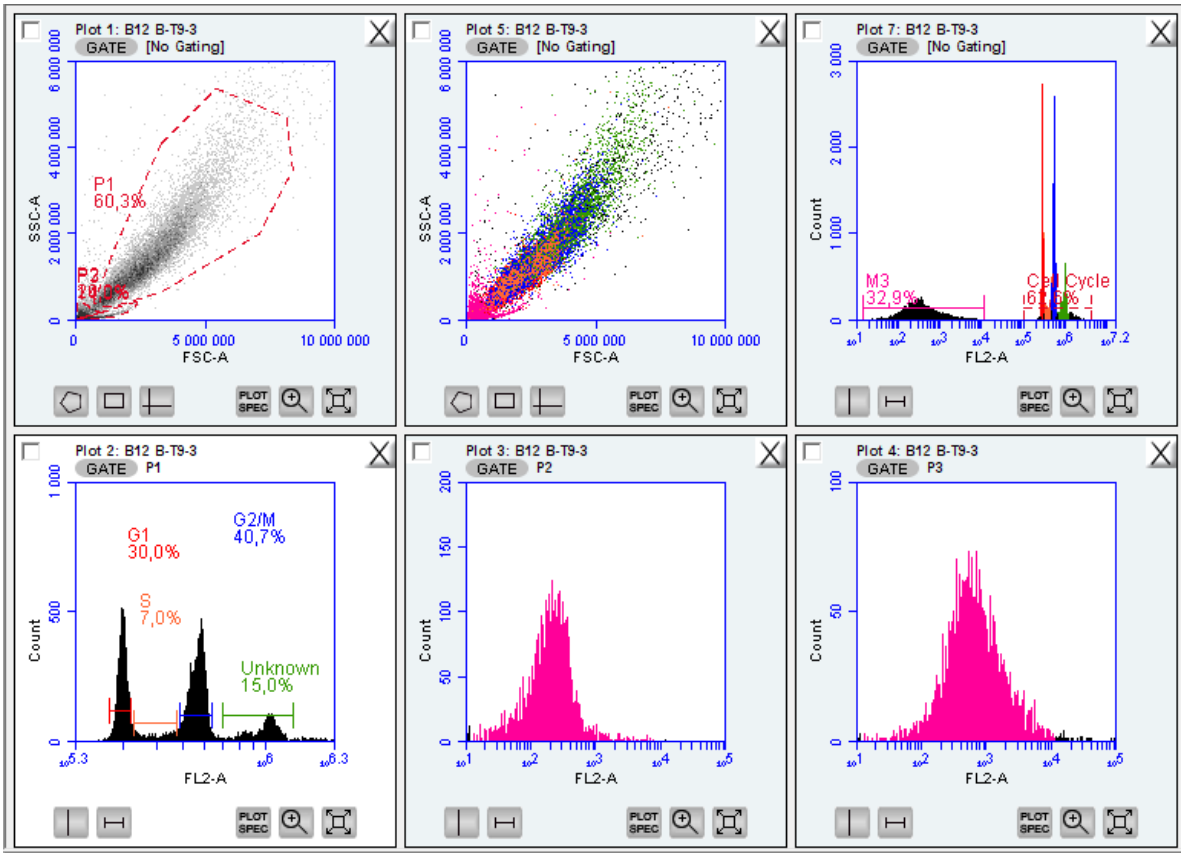
Plot 2: B11 B-T9-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	6 231	263	100,00%	24,16%	469 160,51	60,37%	
G1 (256 419,0 / 32...	2 261	263	36,29%	8,77%	291 581,11	4,26%	
G2/M (486 711,0 / ...	2 185	263	35,07%	8,47%	554 683,76	6,19%	
Unknown (692 838,...	596	263	9,57%	2,31%	964 106,40	13,45%	
S (324 482,0 / 474 ...	492	263	7,90%	1,91%	403 175,83	11,70%	

Plot 3: B11 B-T9-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	8 681	263	100,00%	33,66%	1 024,94	2 874,80%	

Plot 4: B11 B-T9-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	8 542	263	100,00%	33,12%	3 085,77	751,27%	

Figure 120. 26 °C at 42 hours sample Nr. 2.

Appendix C45



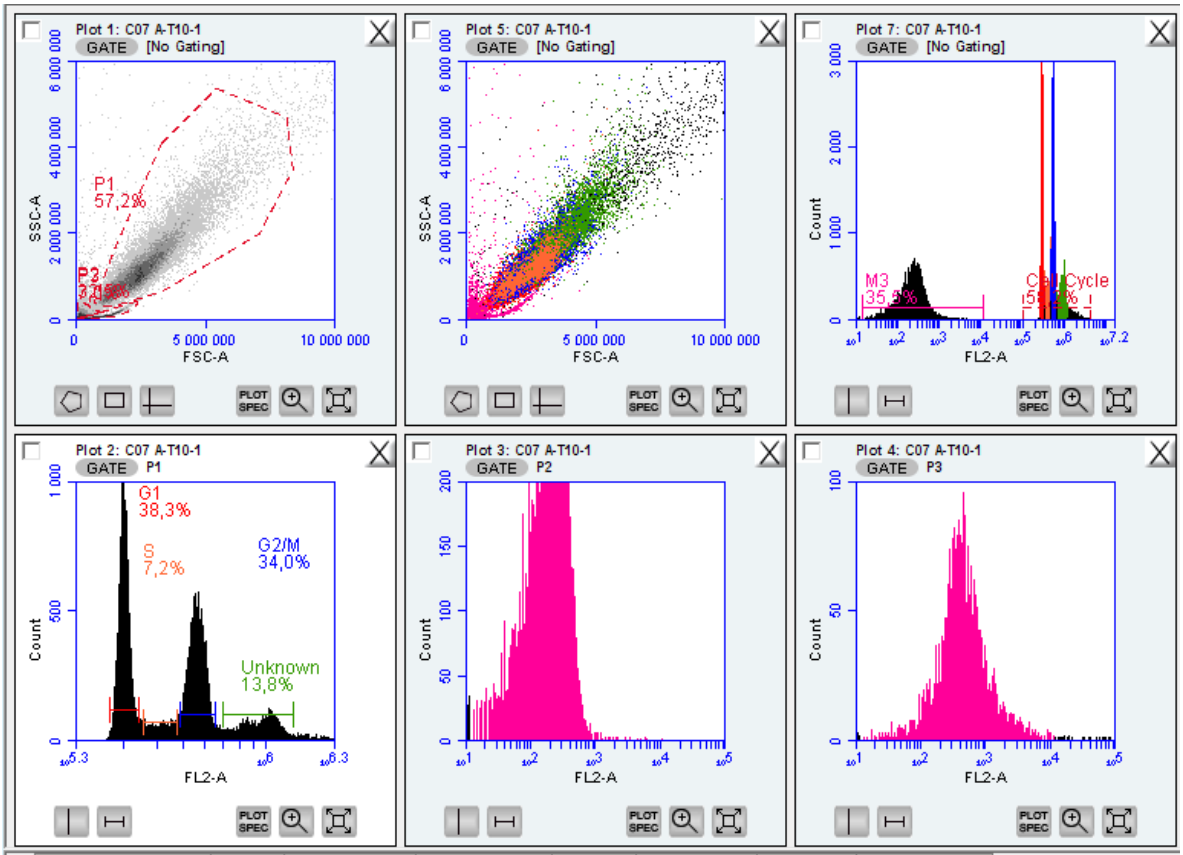
Plot 2: B12 B-T9-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	12 526	254	100,00%	60,30%	557 158,13	56,16%	
G1 (263 215,0 / 32...	3 752	254	29,95%	18,06%	295 985,52	3,70%	
G2/M (486 711,0 / ...	5 093	254	40,66%	24,52%	562 940,14	5,89%	
Unknown (692 838,...	1 879	254	15,00%	9,05%	989 643,60	12,69%	
S (324 482,0 / 474 ...	871	254	6,95%	4,19%	397 374,37	12,04%	

Plot 3: B12 B-T9-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	4 146	254	100,00%	19,96%	1 791,14	1 467,15%	

Plot 4: B12 B-T9-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 903	254	100,00%	13,98%	9 450,05	567,97%	

Figure 121. 26 °C at 42 hours sample Nr. 3.

Appendix C46



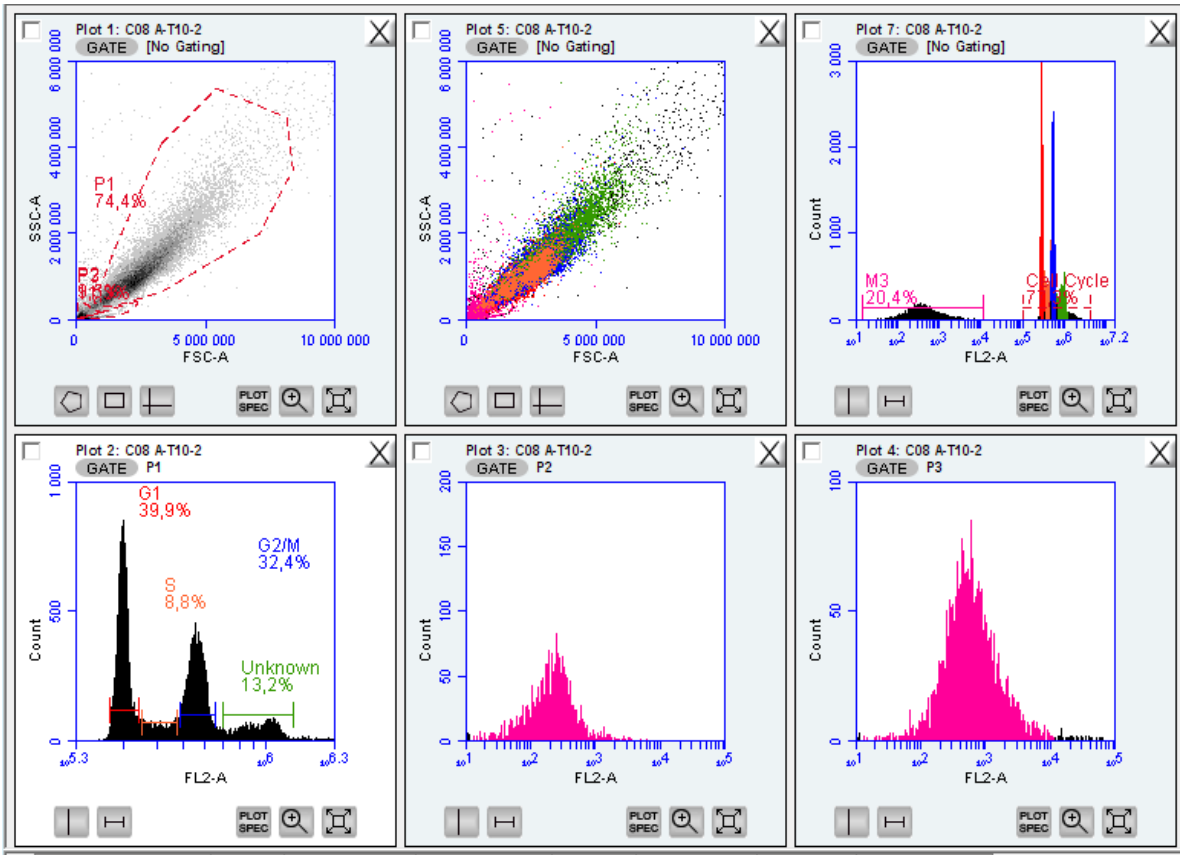
Plot 2: C07 A-T10-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	22 870	254	100,00%	57,18%	531 043,81	58,70%	
G1 (263 215,0 / 341 …	8 766	254	38,33%	21,92%	299 957,68	4,69%	
G2/M (486 711,0 / 6…	7 768	254	33,97%	19,42%	560 857,25	6,56%	
Unknown (692 838,0…	3 149	254	13,77%	7,87%	957 787,64	14,94%	
S (350 971,0 / 474 1…	1 639	254	7,17%	4,10%	412 440,19	9,11%	

Plot 3: C07 A-T10-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	13 007	254	100,00%	32,52%	292,73	1 551,82%	

Plot 4: C07 A-T10-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 800	254	100,00%	7,00%	4 955,24	799,14%	

Figure 122. 20 °C at 52 hours sample Nr. 1.

Appendix C47



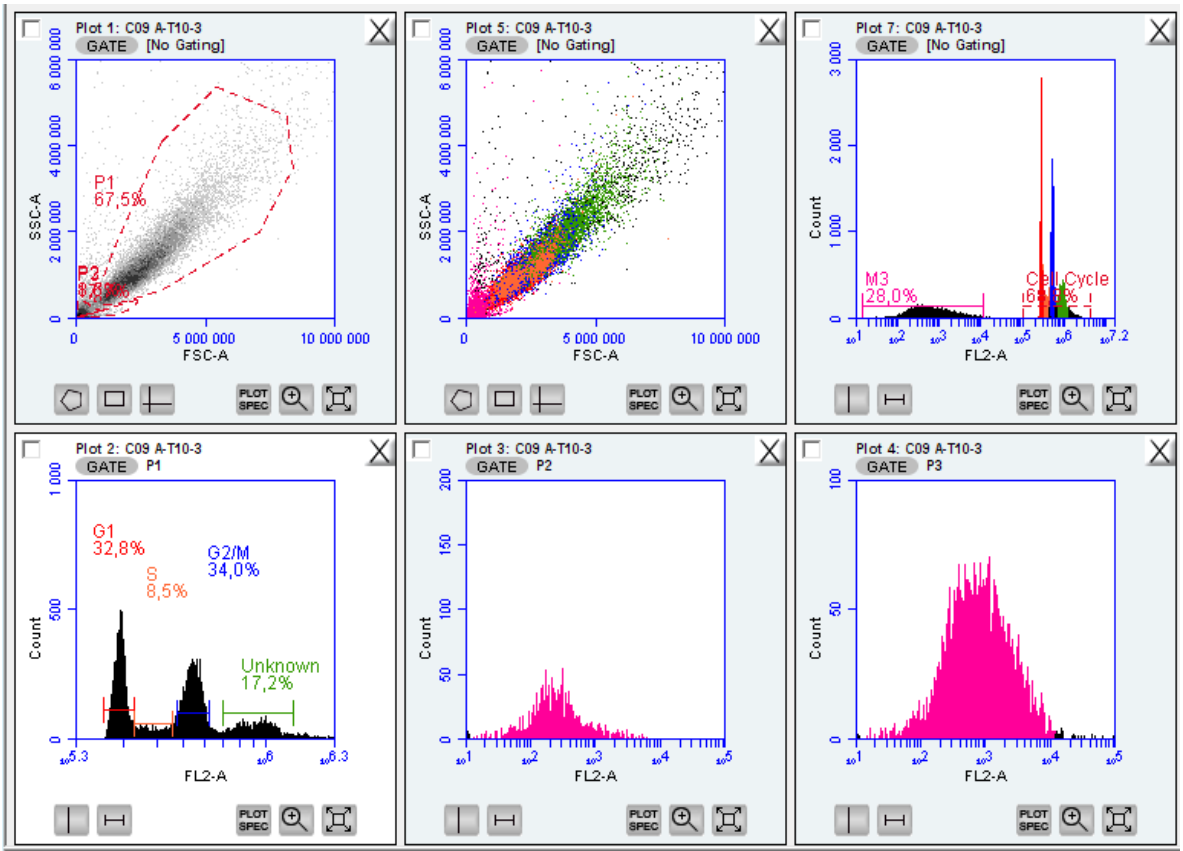
Plot 2: C08 A-T10-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	19 088	223	100,00%	74,41%	508 814,60	55,53%	
G1 (263 215,0 / 341 ...	7 609	223	39,86%	29,66%	297 331,92	5,00%	
G2/M (486 711,0 / 6...	6 188	223	32,42%	24,12%	558 861,93	6,76%	
Unknown (692 838,0...	2 521	223	13,21%	9,83%	944 755,84	15,03%	
S (346 410,0 / 474 1...	1 682	223	8,81%	6,56%	406 915,13	9,58%	

Plot 3: C08 A-T10-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 430	223	100,00%	9,47%	2 309,61	1 187,97%	

Plot 4: C08 A-T10-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 887	223	100,00%	11,25%	16 856,44	409,59%	

Figure 123. 20 °C at 52 hours sample Nr. 2.

Appendix C48



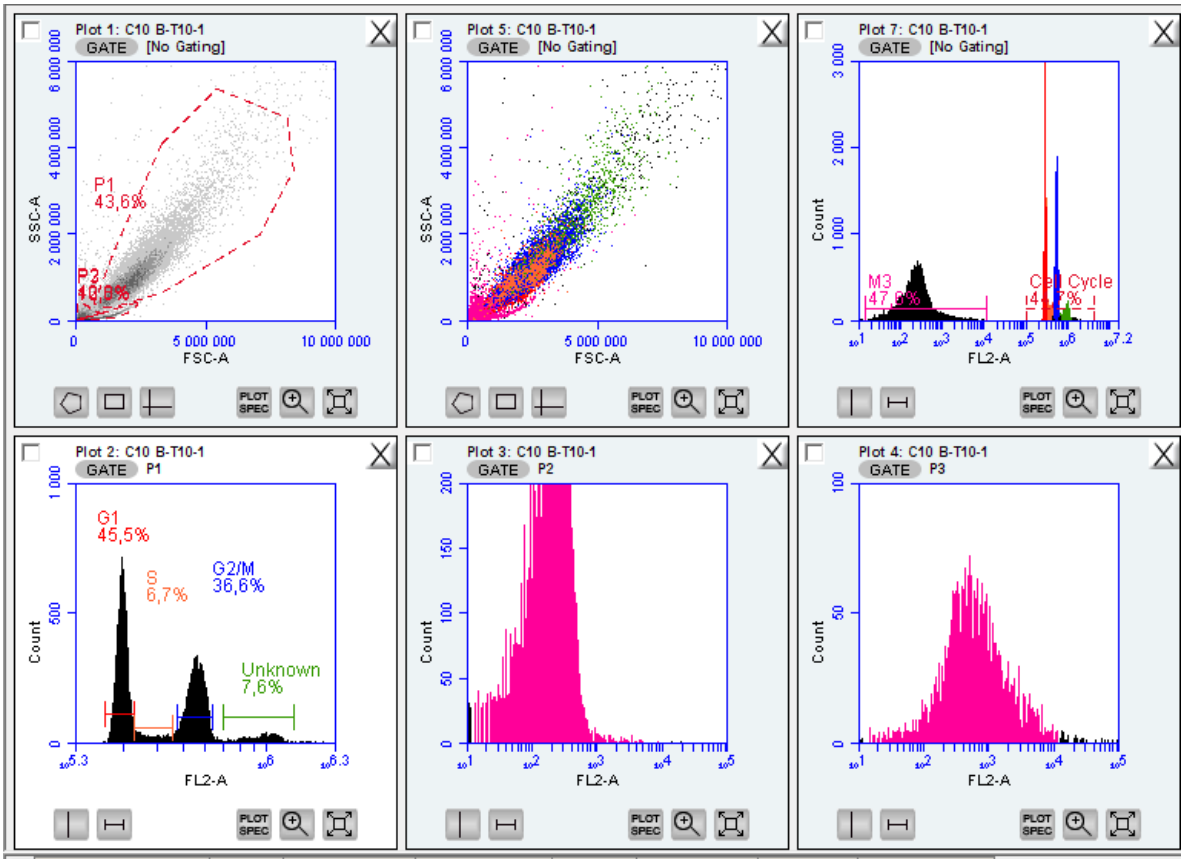
Plot 2: C09 A-T10-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	13 493	132	100,00%	67,46%	543 478,47	57,83%	
G1 (253 087,0 / 328 ...	4 423	132	32,78%	22,12%	289 297,80	4,92%	
G2/M (467 984,0 / 6...	4 585	132	33,98%	22,92%	540 416,72	6,78%	
Unknown (692 838,0...	2 327	132	17,25%	11,64%	936 799,81	15,04%	
S (324 482,0 / 455 9...	1 152	132	8,54%	5,76%	385 216,93	10,15%	

Plot 3: C09 A-T10-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 723	132	100,00%	8,62%	1 157,21	1 581,55%	

Plot 4: C09 A-T10-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 549	132	100,00%	17,74%	4 895,82	667,13%	

Figure 124. 20 °C at 52 hours sample Nr. 3.

Appendix C49



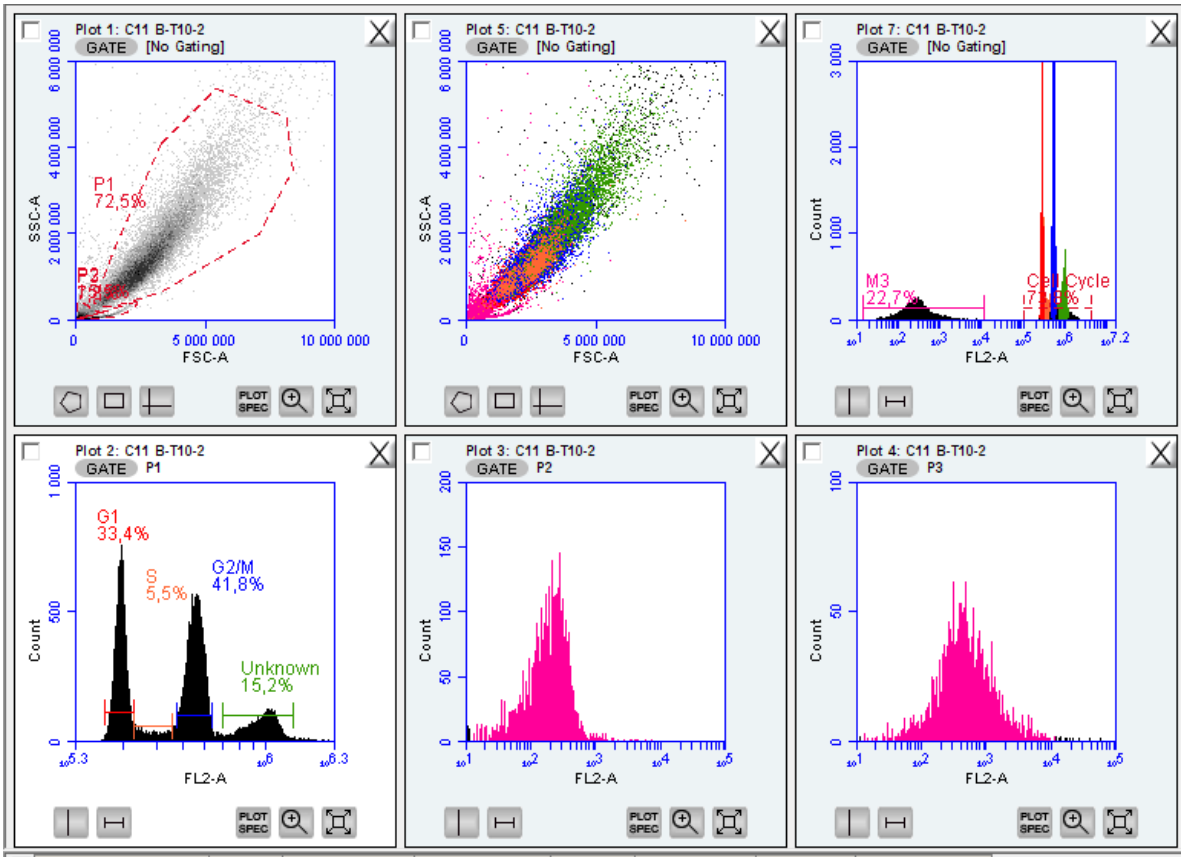
Plot 2: C10 B-T10-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	13 095	234	100,00%	43,65%	460 367,57	51,56%	
G1 (253 087,0 / 328 ...	5 964	234	45,54%	19,88%	294 695,41	4,43%	
G2/M (467 984,0 / 6...	4 790	234	36,58%	15,97%	554 408,45	6,81%	
Unknown (692 838,0...	999	234	7,63%	3,33%	965 980,82	14,09%	
S (324 482,0 / 455 9...	878	234	6,70%	2,93%	379 556,88	10,55%	

Plot 3: C10 B-T10-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	12 827	234	100,00%	42,76%	571,39	2 171,35%	

Plot 4: C10 B-T10-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 015	234	100,00%	10,05%	7 912,39	585,04%	

Figure 125. 26 °C at 52 hours sample Nr. 1.

Appendix C50



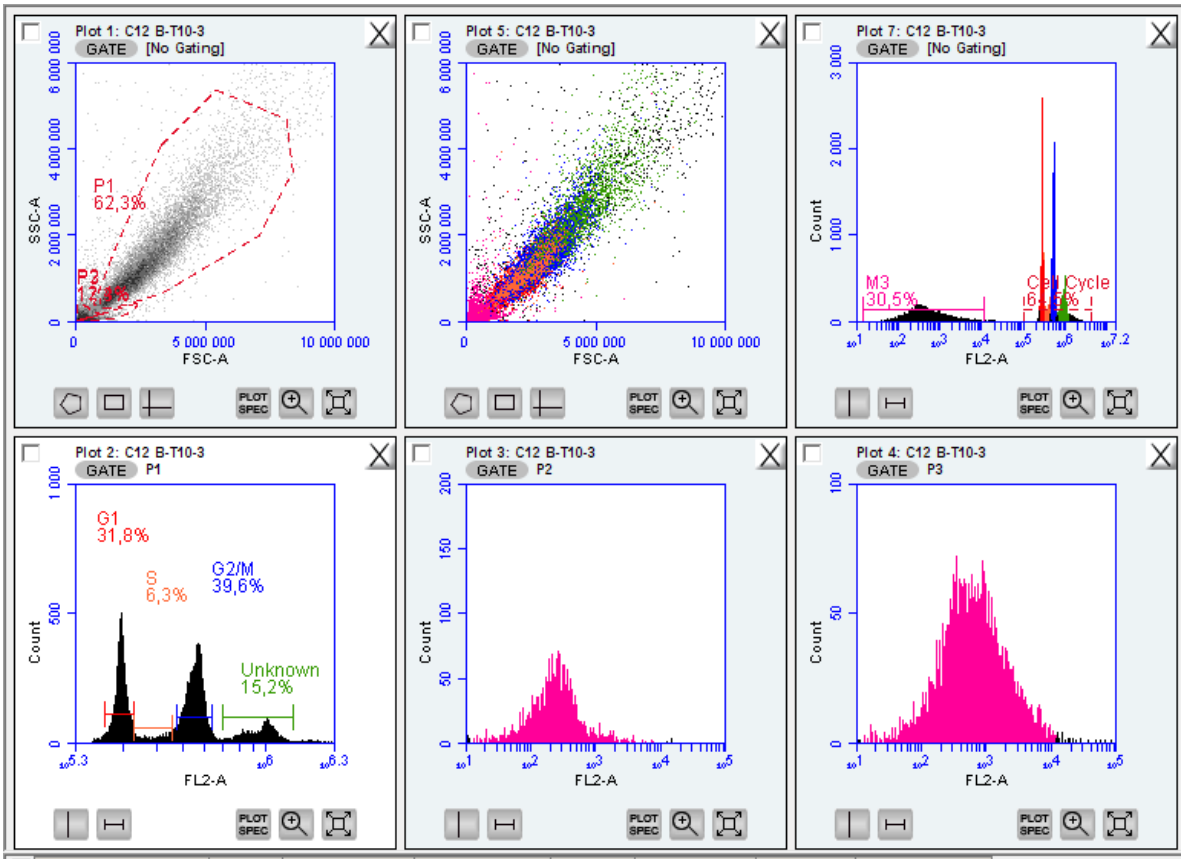
Plot 2: C11 B-T10-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	19 616	142	100,00%	72,54%	528 255,87	51,09%	
G1 (253 087,0 / 328 ...	6 550	142	33,39%	24,22%	291 688,92	4,69%	
G2/M (467 984,0 / 6...	8 198	142	41,79%	30,32%	552 164,43	6,73%	
Unknown (692 838,0...	2 986	142	15,22%	11,04%	971 663,10	13,23%	
S (324 482,0 / 455 9...	1 086	142	5,54%	4,02%	382 937,10	10,49%	

Plot 3: C11 B-T10-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	4 200	142	100,00%	15,53%	479,64	1 870,09%	

Plot 4: C11 B-T10-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 119	142	100,00%	7,84%	9 940,59	547,46%	

Figure 126. 20 °C at 52 hours sample Nr. 2.

Appendix C51



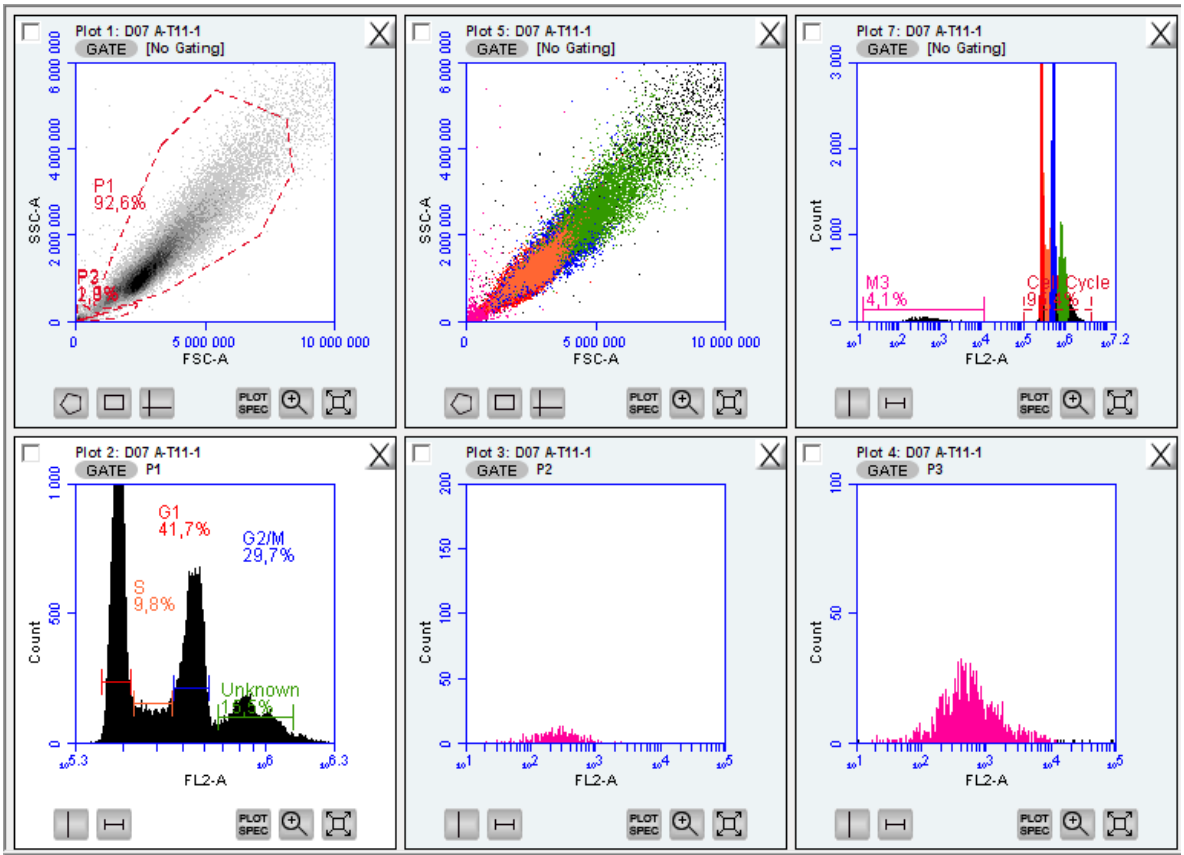
Plot 2: C12 B-T10-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	12 467	366	100,00%	62,33%	526 267,72	53,30%	
G1 (253 087,0 / 328 ...	3 960	366	31,76%	19,80%	292 719,65	4,97%	
G2/M (467 984,0 / 6...	4 933	366	39,57%	24,66%	550 988,33	6,79%	
Unknown (692 838,0...	1 900	366	15,24%	9,50%	961 200,85	13,50%	
S (324 482,0 / 455 9...	785	366	6,30%	3,92%	389 978,36	10,77%	

Plot 3: C12 B-T10-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 478	366	100,00%	12,39%	1 692,08	1 099,33%	

Plot 4: C12 B-T10-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 463	366	100,00%	17,32%	5 057,96	765,79%	

Figure 127. 26 °C at 52 hours sample Nr. 3.

Appendix C52



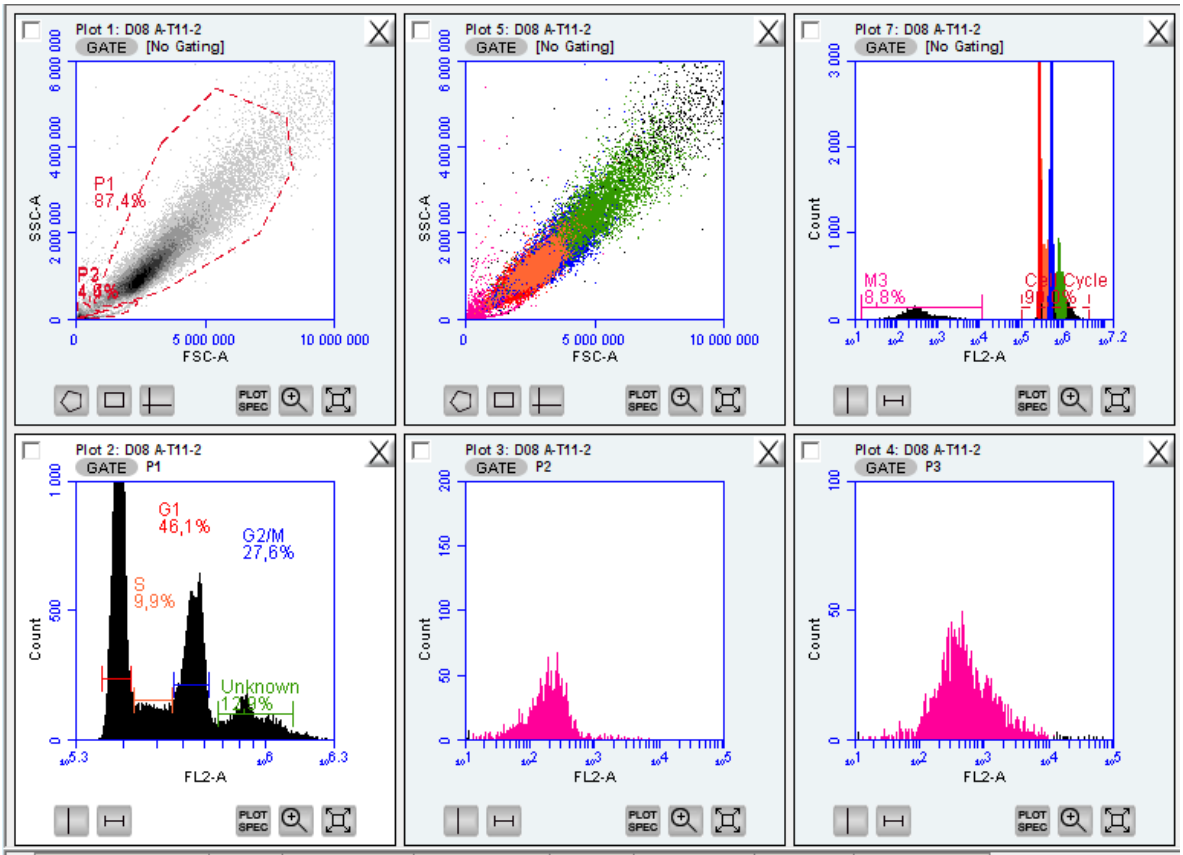
Plot 2: D07 A-T11-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	37 057	68	100,00%	92,64%	484 798,17	51,66%	
G1 (246 553,0 / 320 ...	15 465	68	41,73%	38,66%	285 769,77	4,77%	
G2/M (455 901,0 / 6...	11 017	68	29,73%	27,54%	540 427,20	7,32%	
Unknown (666 180,0...	5 731	68	15,47%	14,33%	904 526,15	16,23%	
S (324 482,0 / 455 9...	3 618	68	9,76%	9,04%	386 995,06	10,15%	

Plot 3: D07 A-T11-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	395	68	100,00%	0,99%	664,14	517,62%	

Plot 4: D07 A-T11-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 122	68	100,00%	2,80%	14 616,78	408,07%	

Figure 128. 20 °C at 56 hours sample Nr. 1.

Appendix C53



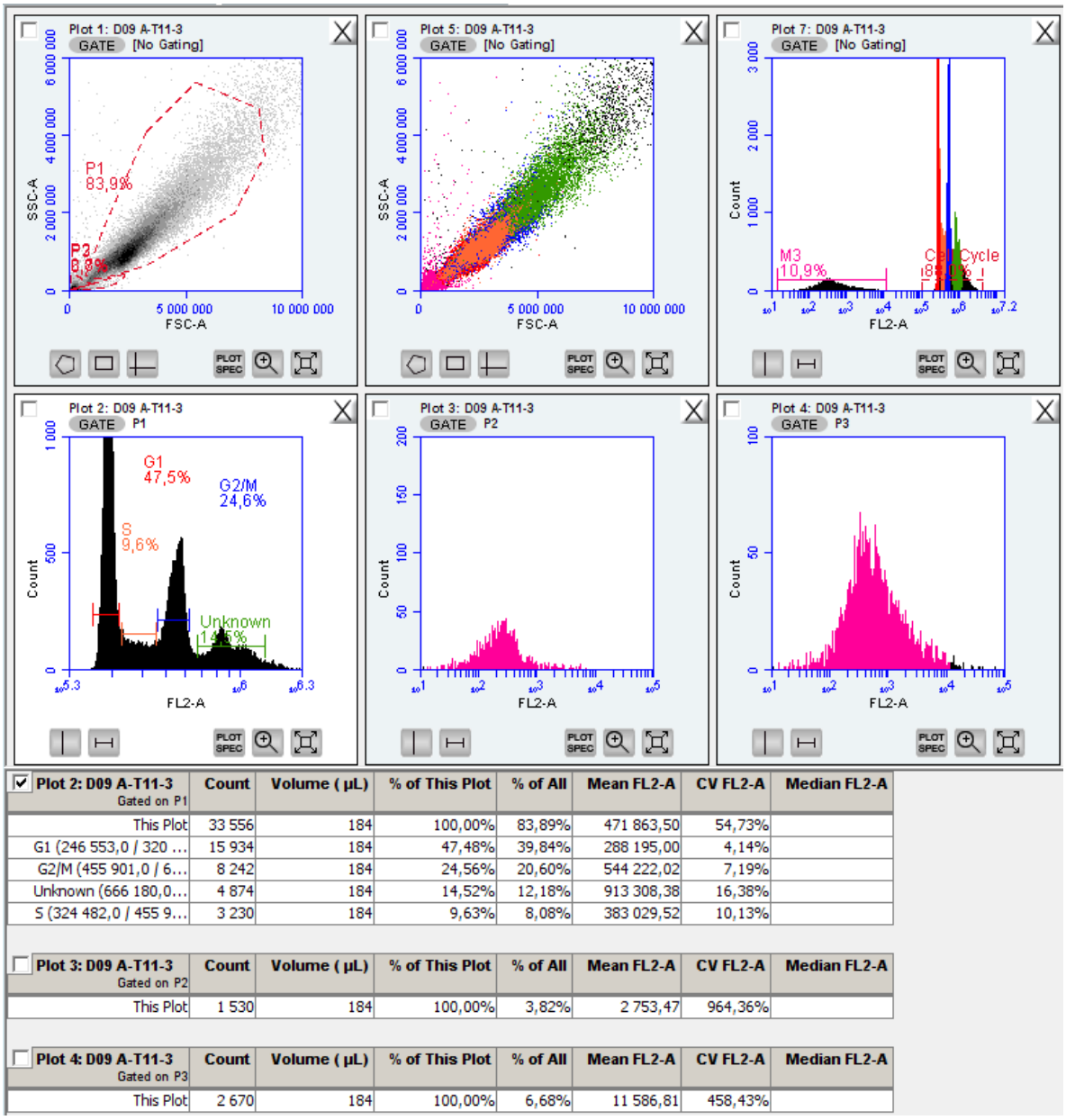
Plot 2: D08 A-T11-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	34 963	119	100,00%	87,41%	463 617,46	52,13%	
G1 (246 553,0 / 320 ...	16 107	119	46,07%	40,27%	286 848,19	4,59%	
G2/M (455 901,0 / 6...	9 633	119	27,55%	24,08%	542 013,51	7,31%	
Unknown (666 180,0...	4 525	119	12,94%	11,31%	905 010,00	16,41%	
S (324 482,0 / 455 9...	3 477	119	9,94%	8,69%	385 553,06	10,00%	

Plot 3: D08 A-T11-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 838	119	100,00%	4,60%	1 403,61	1 475,30%	

Plot 4: D08 A-T11-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 742	119	100,00%	4,35%	12 827,55	450,25%	

Figure 129. 20 °C at 56 hours sample Nr. 2.

Appendix C54



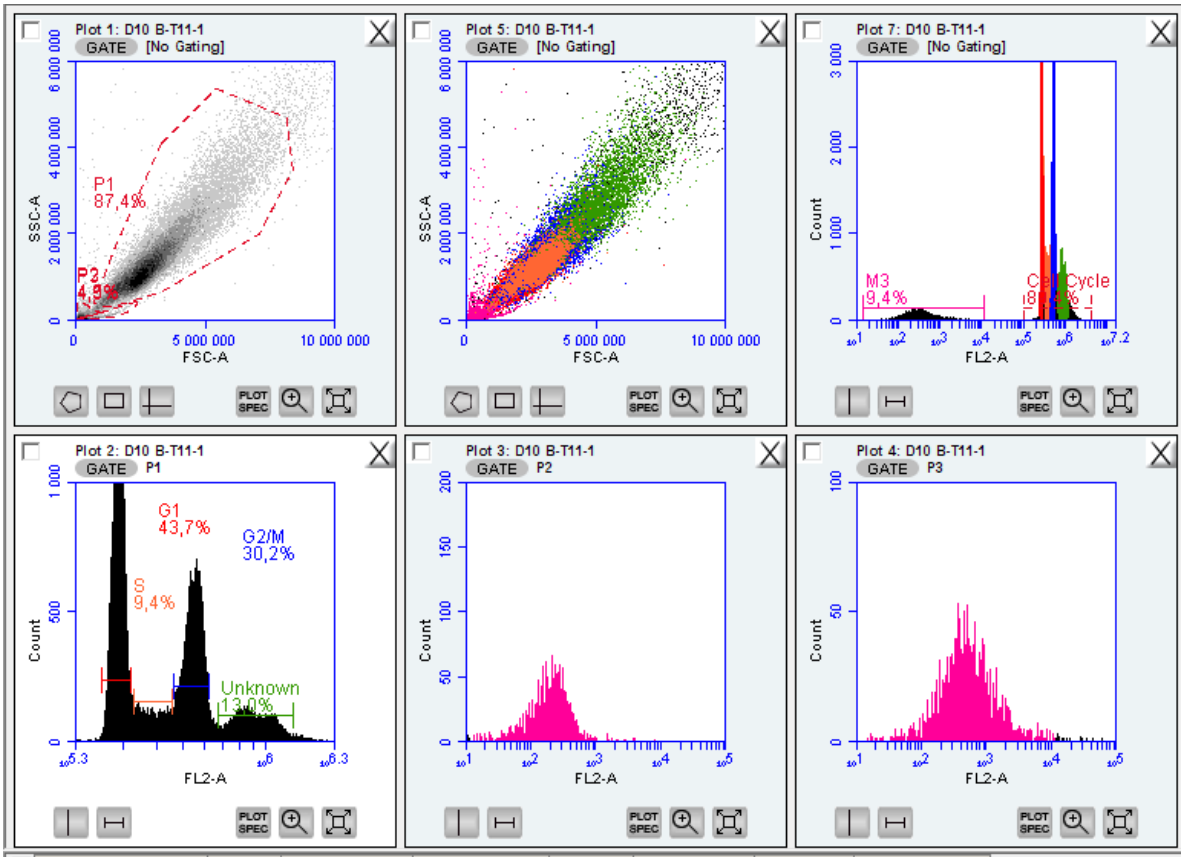
Plot 2: D09 A-T11-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	33 556	184	100,00%	83,89%	471 863,50	54,73%	
G1 (246 553,0 / 320 ...	15 934	184	47,48%	39,84%	288 195,00	4,14%	
G2/M (455 901,0 / 6...	8 242	184	24,56%	20,60%	544 222,02	7,19%	
Unknown (666 180,0...	4 874	184	14,52%	12,18%	913 308,38	16,38%	
S (324 482,0 / 455 9...	3 230	184	9,63%	8,08%	383 029,52	10,13%	

Plot 3: D09 A-T11-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 530	184	100,00%	3,82%	2 753,47	964,36%	

Plot 4: D09 A-T11-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 670	184	100,00%	6,68%	11 586,81	458,43%	

Figure 130. 20 °C at 56 hours sample Nr. 1.

Appendix C55



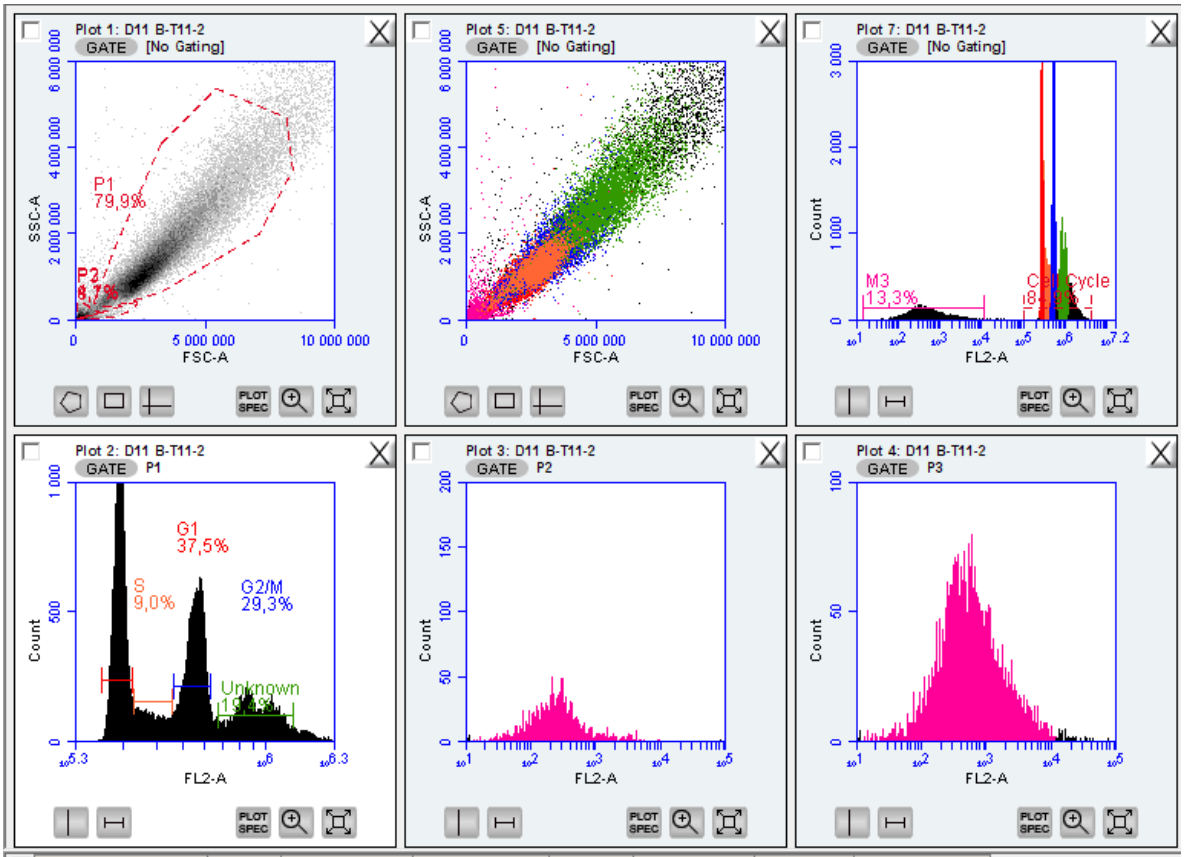
Plot 2: D10 B-T11-1 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	34 955	93	100,00%	87,39%	469 407,45	51,36%	
G1 (246 553,0 / 320 ...	15 291	93	43,74%	38,23%	286 426,77	4,81%	
G2/M (455 901,0 / 6...	10 541	93	30,16%	26,35%	543 435,76	7,21%	
Unknown (666 180,0...	4 538	93	12,98%	11,34%	915 454,20	16,17%	
S (324 482,0 / 455 9...	3 274	93	9,37%	8,18%	385 297,26	10,14%	

Plot 3: D10 B-T11-1 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 956	93	100,00%	4,89%	2 916,31	1 189,83%	

Plot 4: D10 B-T11-1 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 819	93	100,00%	4,55%	12 395,09	445,57%	

Figure 131. 26 °C at 56 hours sample Nr. 1.

Appendix C56



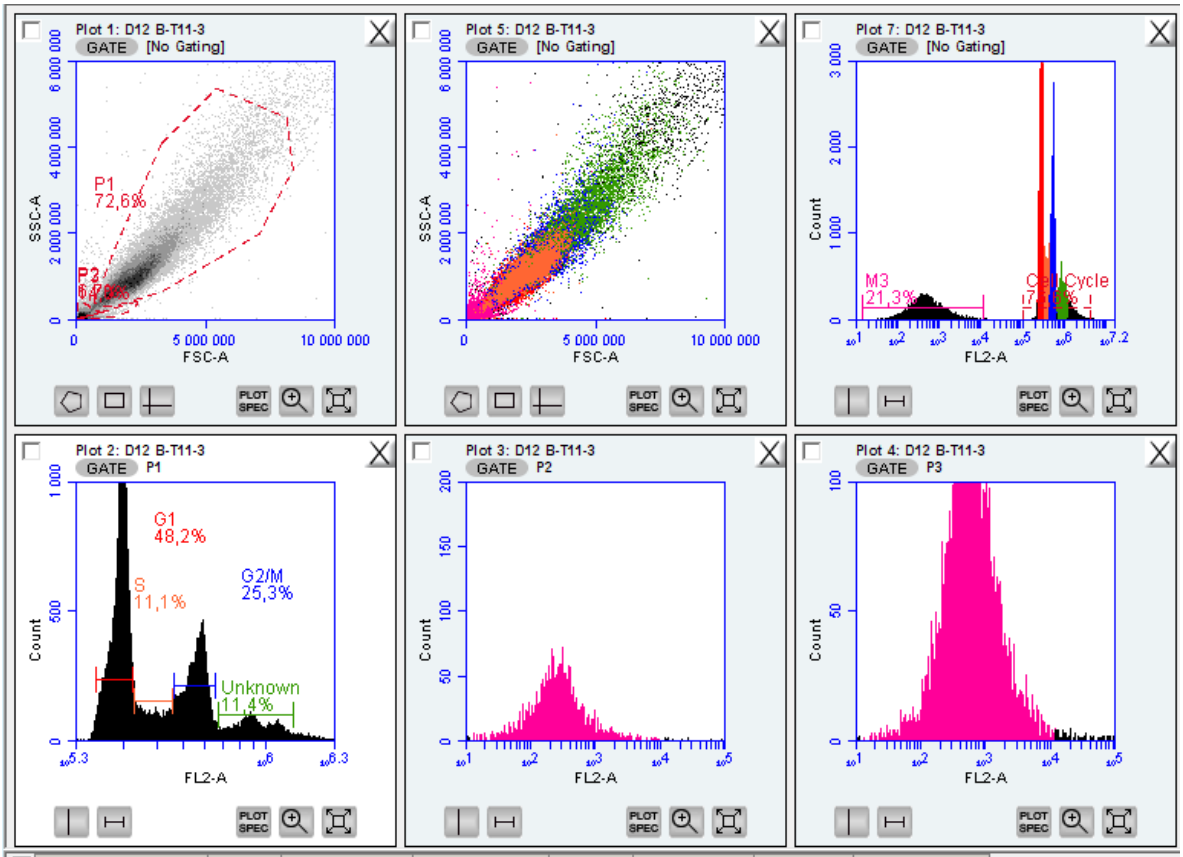
Plot 2: D11 B-T11-2 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	31 961	155	100,00%	79,90%	529 614,70	55,28%	
G1 (246 553,0 / 324 ...	11 976	155	37,47%	29,94%	289 188,34	4,76%	
G2/M (455 901,0 / 6...	9 362	155	29,29%	23,40%	550 380,29	7,31%	
Unknown (666 180,0...	6 216	155	19,45%	15,54%	934 878,54	16,47%	
S (324 482,0 / 455 9...	2 880	155	9,01%	7,20%	381 351,81	10,01%	

Plot 3: D11 B-T11-2 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	1 625	155	100,00%	4,06%	4 174,95	904,39%	

Plot 4: D11 B-T11-2 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	3 477	155	100,00%	8,69%	9 707,89	508,92%	

Figure 132. 26 °C at 56 hours sample Nr. 2.

Appendix C57



Plot 2: D12 B-T11-3 Gated on P1	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	29 059	442	100,00%	72,65%	462 552,86	59,07%	
G1 (233 986,0 / 324 ...	14 018	442	48,24%	35,04%	287 538,79	7,06%	
G2/M (455 901,0 / 6...	7 361	442	25,33%	18,40%	553 353,14	8,72%	
Unknown (666 180,0...	3 319	442	11,42%	8,30%	929 991,92	16,82%	
S (324 482,0 / 455 9...	3 224	442	11,09%	8,06%	383 242,46	10,45%	

Plot 3: D12 B-T11-3 Gated on P2	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	2 664	442	100,00%	6,66%	6 806,60	667,86%	

Plot 4: D12 B-T11-3 Gated on P3	Count	Volume (µL)	% of This Plot	% of All	Mean FL2-A	CV FL2-A	Median FL2-A
This Plot	5 613	442	100,00%	14,03%	9 108,34	514,83%	

Figure 133. 26 °C at 56 hours sample Nr. 3.

Appendix D1

20°C	% of P1									% of all												
Phase	G1			S			G2/M			G1			S			G2/M			Debris		
Parallel	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
0	29	35	44	14	14	16	35	35	35	25	29	35	12	12	12	30	29	23	11	14	18
2	33		42	11		11	37		31	30		35	10		10	33		26	6		13
4	49	60		13	11		29	24		35	40		10	7		30	16		25	29	
6	46	61	47	10	9	9	30	23	30	41	46	41	9	7	8	27	18	27	9	20	7
8	28	34	39	4	4	4	43	42	39	22	28	30	3	4	3	34	35	29	14	13	20
26	41	52	39	12	15	12	30	26	30	25	32	15	7	9	4	19	16	11	34	34	53
30	32	40	32	5	6	7	40	39	39	28	36	24	4	5	5	34	35	29	11	8	19
42	33	49	45	15	13	14	34	27	25	11	22	9	5	6	3	11	12	5	55	49	70
52	38	40	33	7	9	9	34	32	34	22	30	22	4	7	6	19	24	23	36	20	28
56	42	46	47	10	10	10	30	28	25	39	40	40	9	9	8	28	24	21	4	9	11

26°C	% of P1									% of all												
Phase	G1			S			G2/M			G1			S			G2/M			Debris		
Parallel	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
0	34	38	46	15	15	17	34	32	28	29	33	23	13	14	8	29	28	14	10	10	42
2		39	43		14	17		32	26		36	34		12	14		29	21		6	16
4	59	61	48	7	7	6	26	22	33	30	17	20	3	2	3	13	6	14	42	63	51
6	58	64	53	6	6	6	28	24	32	51	45	44	5	4	5	25	17	26	10	25	14
8	34	40	29	4	4	5	42	43	43	24	29	18	3	3	3	29	31	27	25	22	30
26	24	16	28	22	22	29	33	29	24	13	9	5	12	12	6	19	16	5	38	35	71
30	22	30	35	11	12	12	39	37	37	18	23	21	9	9	7	32	28	22	14	21	34
42	38	36	30	8	8	7	36	35	41	20	9	18	5	2	4	20	8	25	40	68	33
52	46	33	32	7	6	6	37	42	40	20	24	20	3	4	4	16	30	25	48	23	31
56	44	37	48	9	9	11	30	29	25	38	30	35	8	7	8	26	23	18	9	13	21

Figure 134. Table of raw data from flow cytometer.

Appendix D2

20°C	% of P1						% of all							
	G1		S		G2/M		G1		S		G2/M		Debris	
Hours	Avg.	Std Dev	Avg	Std Dev	Avg.	Std Dev	Avg.	Std Dev	Avg.	Std Dev	Avg.	Std Dev	Avg.	Std Dev
0	36	8	15	1	35	1	30	5	12	0	27	4	14	3
2	38	6	11	0	34	4	33	4	10	0	30	5	9	5
4	55	8	12	2	26	4	37	4	8	2	23	10	27	3
6	51	8	9	1	28	4	43	3	8	1	24	5	12	7
8	34	6	4	0	41	2	27	4	3	0	33	3	16	4
26	44	7	13	2	29	3	24	9	7	2	15	4	40	11
30	35	4	6	1	39	0	29	6	5	1	33	3	12	6
42	42	9	14	1	28	5	14	7	5	2	9	4	58	11
52	37	4	8	1	33	1	25	4	5	1	22	2	28	8
56	45	3	10	0	27	3	40	1	9	0	24	3	8	3

26°C	% of P1						% of all							
	G1		S		G2/M		G1		S		G2/M		Debris	
Hours	Avg.	Std Dev	Avg	Std Dev	Avg.	Std Dev	Avg.	Std Dev	Avg.	Std Dev	Avg.	Std Dev	Avg.	Std Dev
0	39	6	16	1	31	3	28	5	12	3	24	9	21	18
2	41	3	15	2	29	4	35	1	13	1	25	6	11	7
4	56	7	7	0	27	6	22	7	3	1	11	4	52	10
6	58	6	6	1	28	4	47	4	5	1	23	5	16	8
8	34	6	4	1	42	1	24	5	3	0	29	2	26	4
26	22	6	24	4	29	5	9	4	10	4	13	7	48	20
30	29	7	12	0	38	1	21	2	8	1	27	5	23	10
42	35	4	8	1	37	3	16	6	4	1	18	8	47	19
52	37	8	6	1	39	3	21	3	4	1	24	7	34	13
56	43	5	10	1	28	3	34	4	8	1	23	4	15	6

Figure 135. Table of raw data averages and standard deviation.

Appendix D3

20°C G1 Hours	% of P1 Avg.	Std Dev	σ+1	σ-1
0	36	8	44	28
2	38	6	44	31
4	55	8	63	47
6	51	8	60	43
8	34	6	40	28
26	44	7	51	37
30	35	4	39	30
42	42	9	51	34
52	37	4	41	33
56	45	3	48	42

Figure 136. Table of averages and standard deviation for G1 phase at 20 °C for P1.

Appendix D4

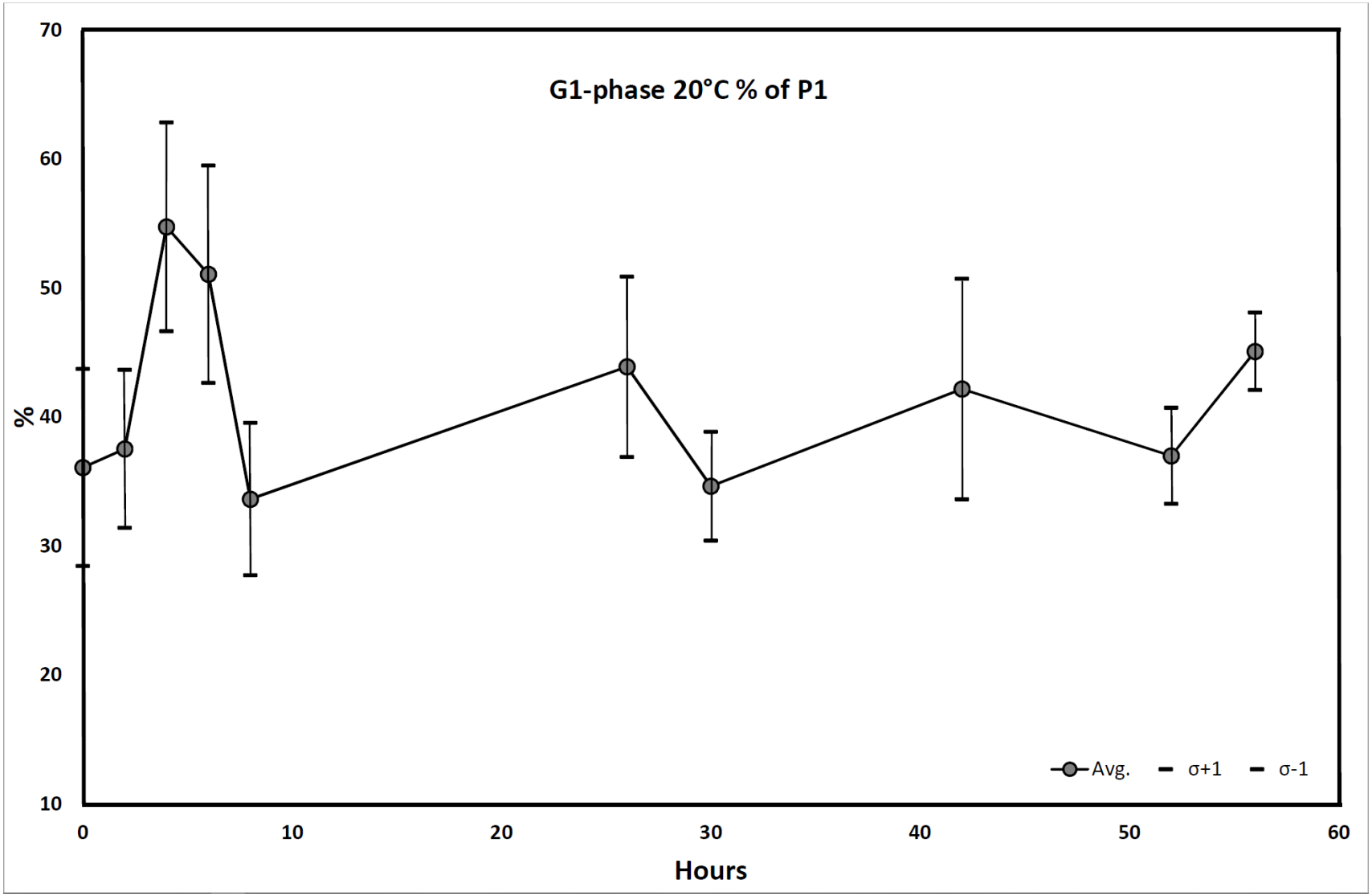


Figure 137. Chart of averages and standard deviation for G1 phase at 20 °C for P1.

Appendix D5

20°C S Hours	% of P1 Avg.	Std Dev	σ+1	σ-1
0	15	1	15	14
2	11	0	11	11
4	12	2	14	10
6	9	1	10	8
8	4	0	4	4
26	13	2	14	11
30	6	1	7	5
42	14	1	15	13
52	8	1	9	7
56	10	0	10	10

Figure 138. Table of averages and standard deviation for S phase at 20 °C for P1.

Appendix D6

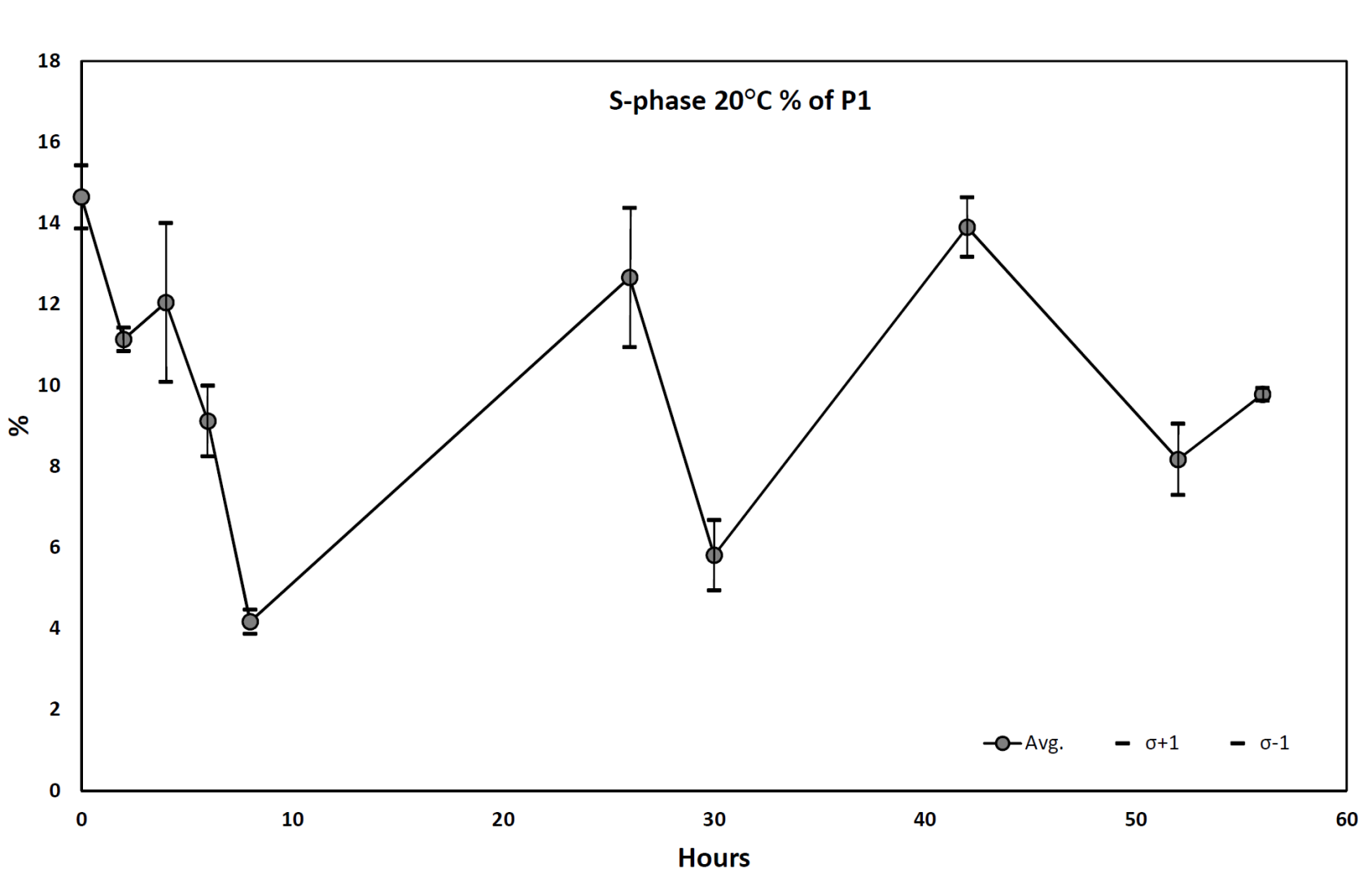


Figure 139. Chart of averages and standard deviation for S phase at 20 °C for P1.

Appendix D7

20°C G2/M Hours	% of P1 Avg.	Std Dev	σ+1	σ-1
0	35	1	35	34
2	34	4	38	30
4	26	4	30	22
6	28	4	32	24
8	41	2	43	40
26	29	3	31	26
30	39	0	40	39
42	28	5	33	24
52	33	1	34	33
56	27	3	30	25

Figure 140. Table of averages and standard deviation for G2/M phase at 20 °C for P1.

Appendix D8

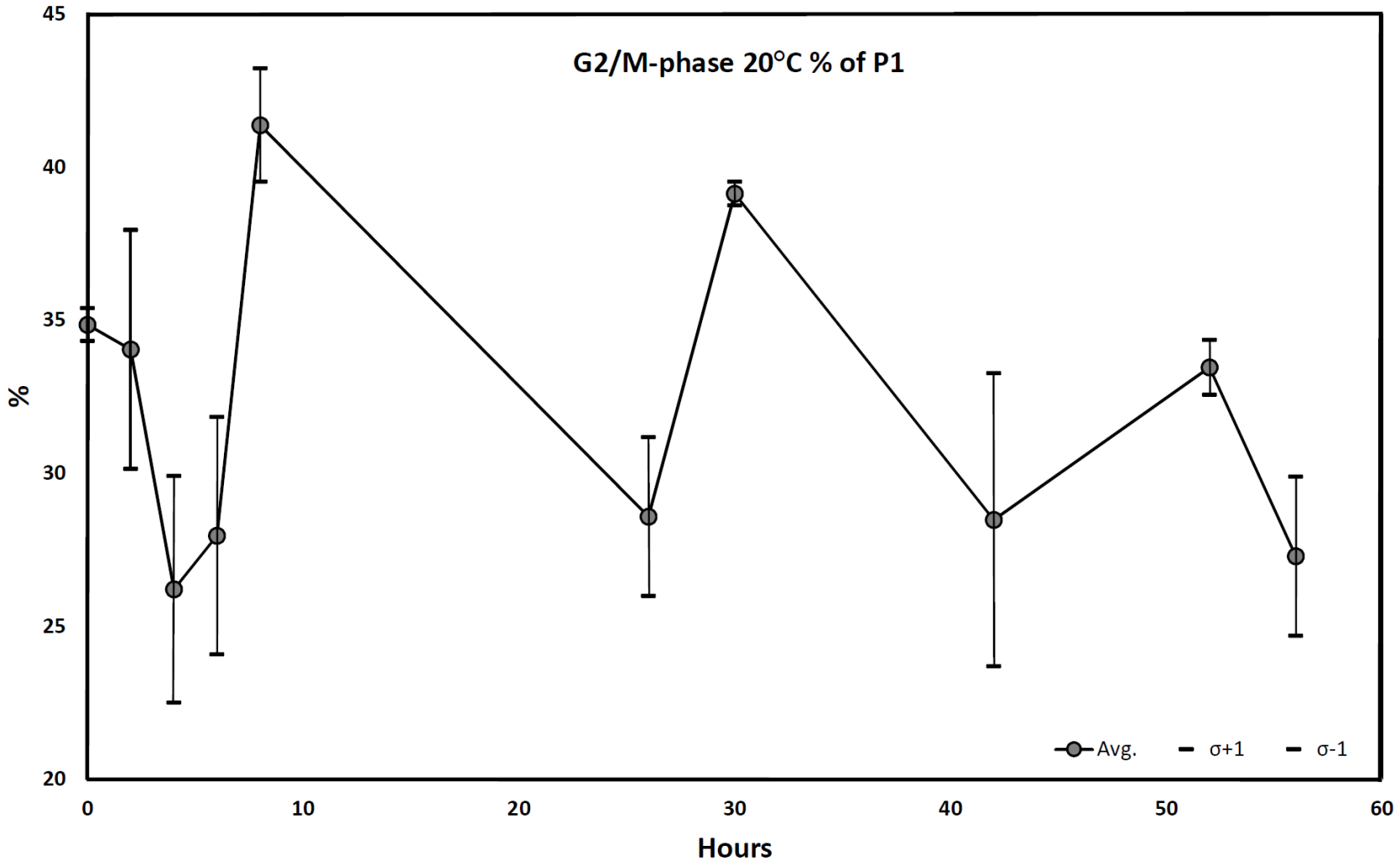


Figure 141. Chart of averages and standard deviation for G2/M phase at 20 °C for P1.

Appendix D9

20°C G1 Hours	% of all Avg.	Std Dev	σ+1	σ-1
0	30	5	35	25
2	33	4	36	29
4	37	4	41	34
6	43	3	45	40
8	27	4	30	23
26	24	9	32	15
30	29	6	35	23
42	14	7	21	7
52	25	4	29	20
56	40	1	40	39

Figure 142. Table of averages and standard deviation for G1 phase at 20 °C for all.

Appendix D10

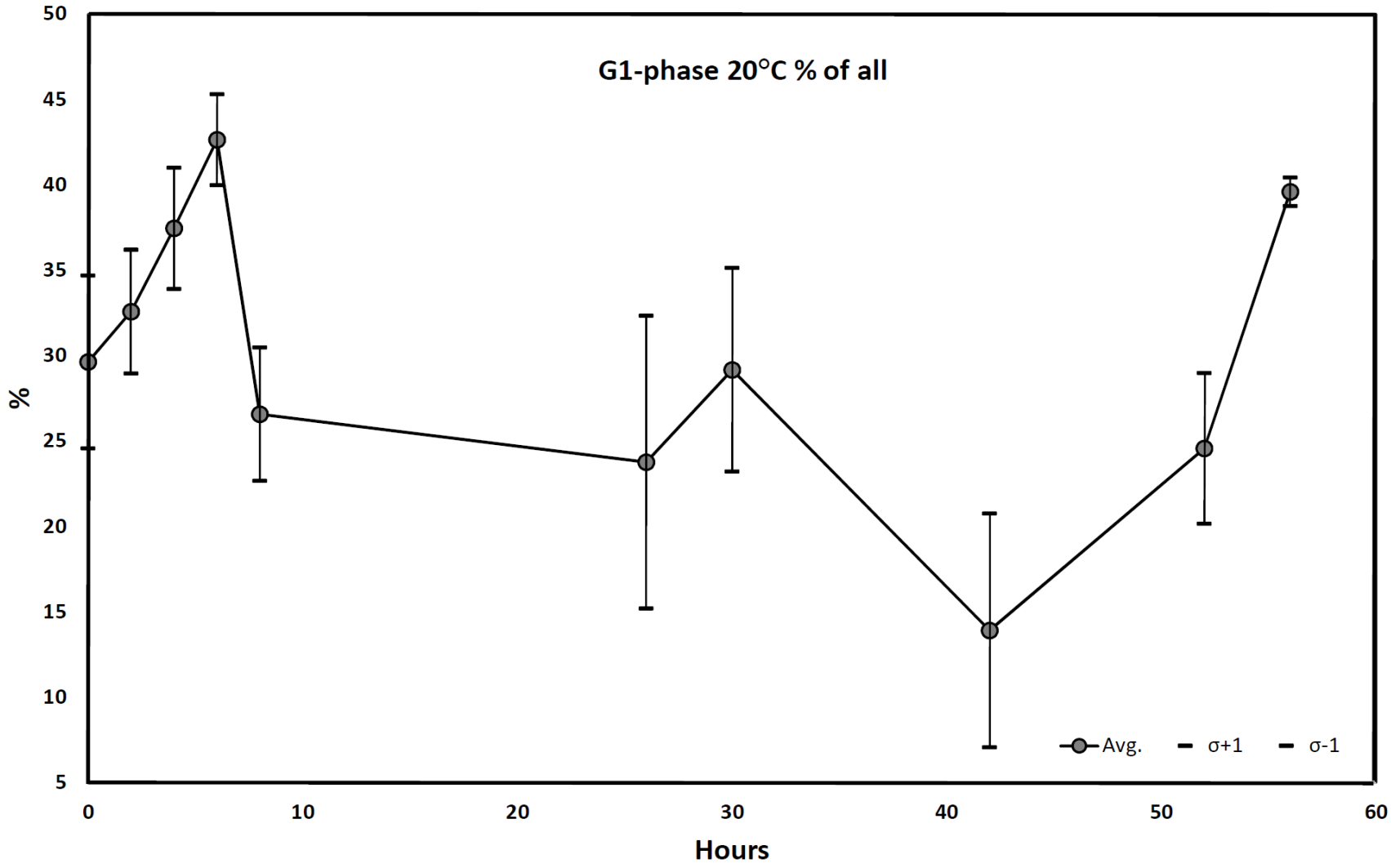


Figure 143. Chart of averages and standard deviation for G1 phase at 20 °C for all.

Appendix D11

20°C S Hours	% of all Avg.	Std Dev	σ+1	σ-1
0	12	0	12	12
2	10	0	10	9
4	8	2	10	7
6	8	1	9	6
8	3	0	4	3
26	7	2	9	5
30	5	1	5	4
42	5	2	6	3
52	5	1	7	4
56	9	0	9	8

Figure 144. Table of averages and standard deviation for S phase at 20 °C for all.

Appendix D12

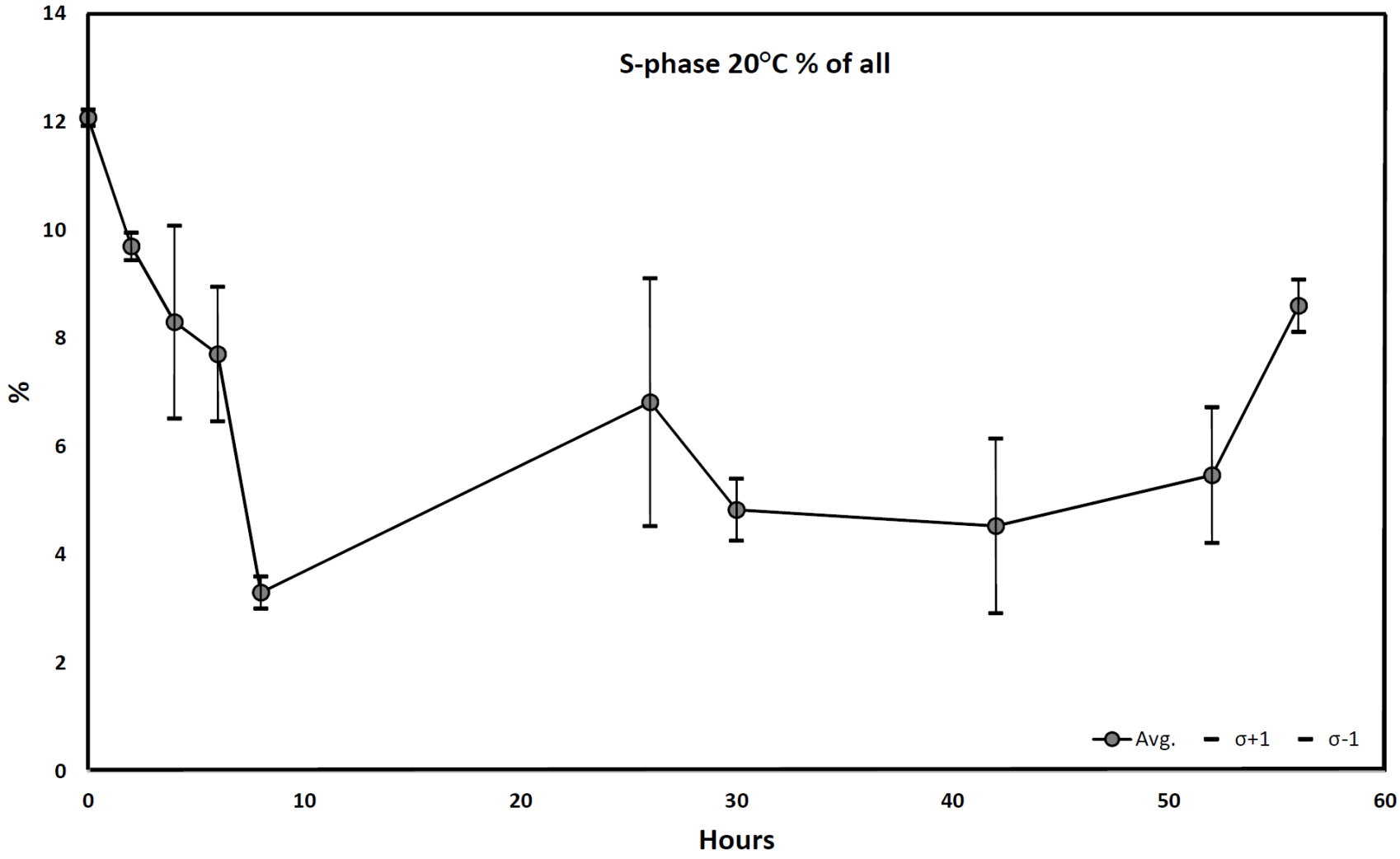


Figure 145. Chart of averages and standard deviation for S phase at 20 °C for all.

Appendix D13

20°C G2/M Hours	% of all Avg.	Std Dev	σ+1	σ-1
0	27	4	31	23
2	30	5	35	25
4	23	10	32	13
6	24	5	29	18
8	33	3	36	30
26	15	4	19	11
30	33	3	36	30
42	9	4	13	6
52	22	2	25	20
56	24	3	28	21

Figure 146. Table of averages and standard deviation for G2/M phase at 20 °C for all.

Appendix D14

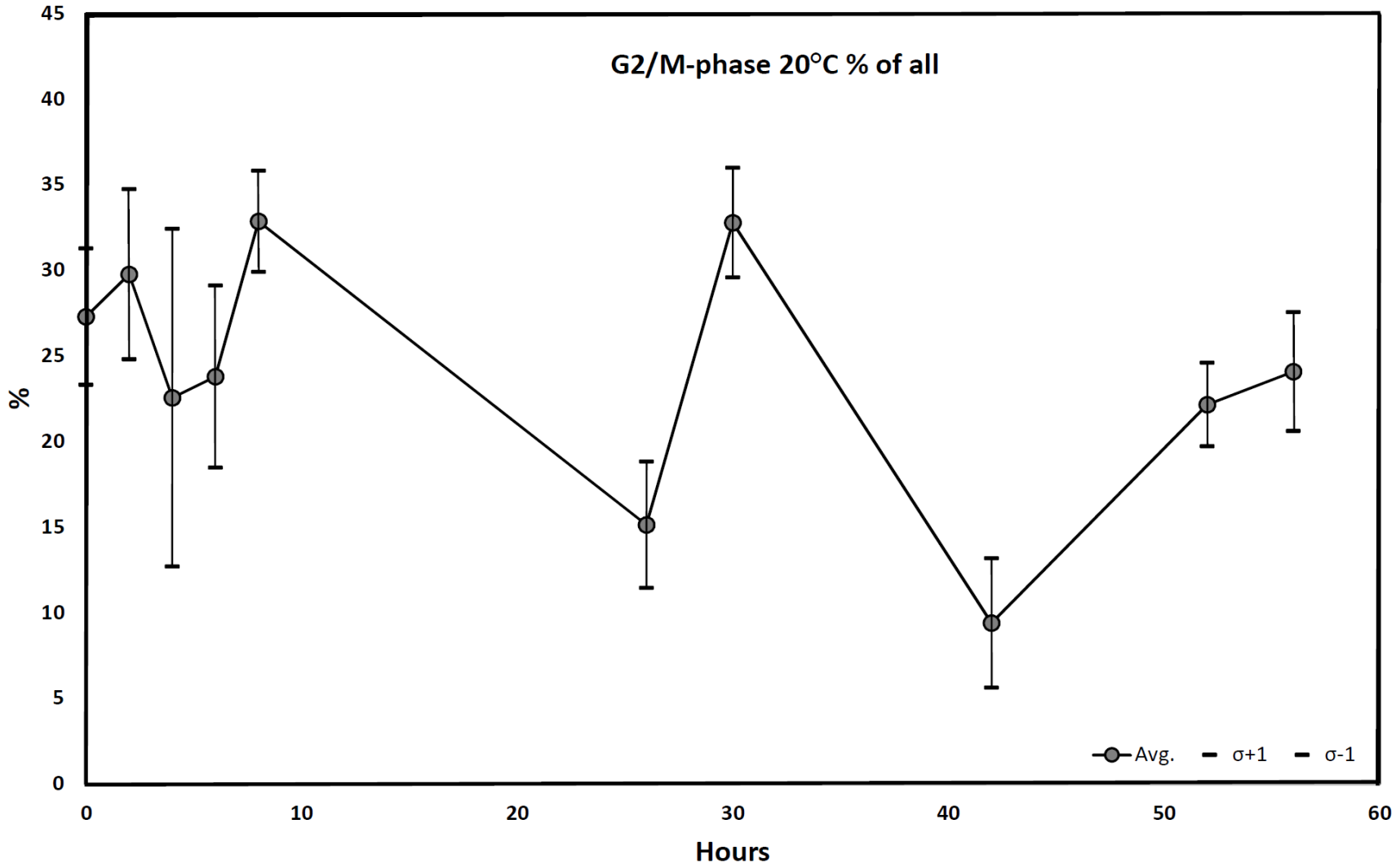


Figure 147. Chart of averages and standard deviation for G2/M phase at 20 °C for all.

Appendix D15

20°C Debris Hours	% of all Avg.	Std Dev	σ+1	σ-1
0	14	3	17	11
2	9	5	15	4
4	27	3	30	24
6	12	7	19	5
8	16	4	19	12
26	40	11	51	29
30	12	6	18	6
42	58	11	69	47
52	28	8	36	20
56	8	3	11	4

Figure 148. Table of averages and standard deviation for cellular debris at 20 °C for all.

Appendix D16

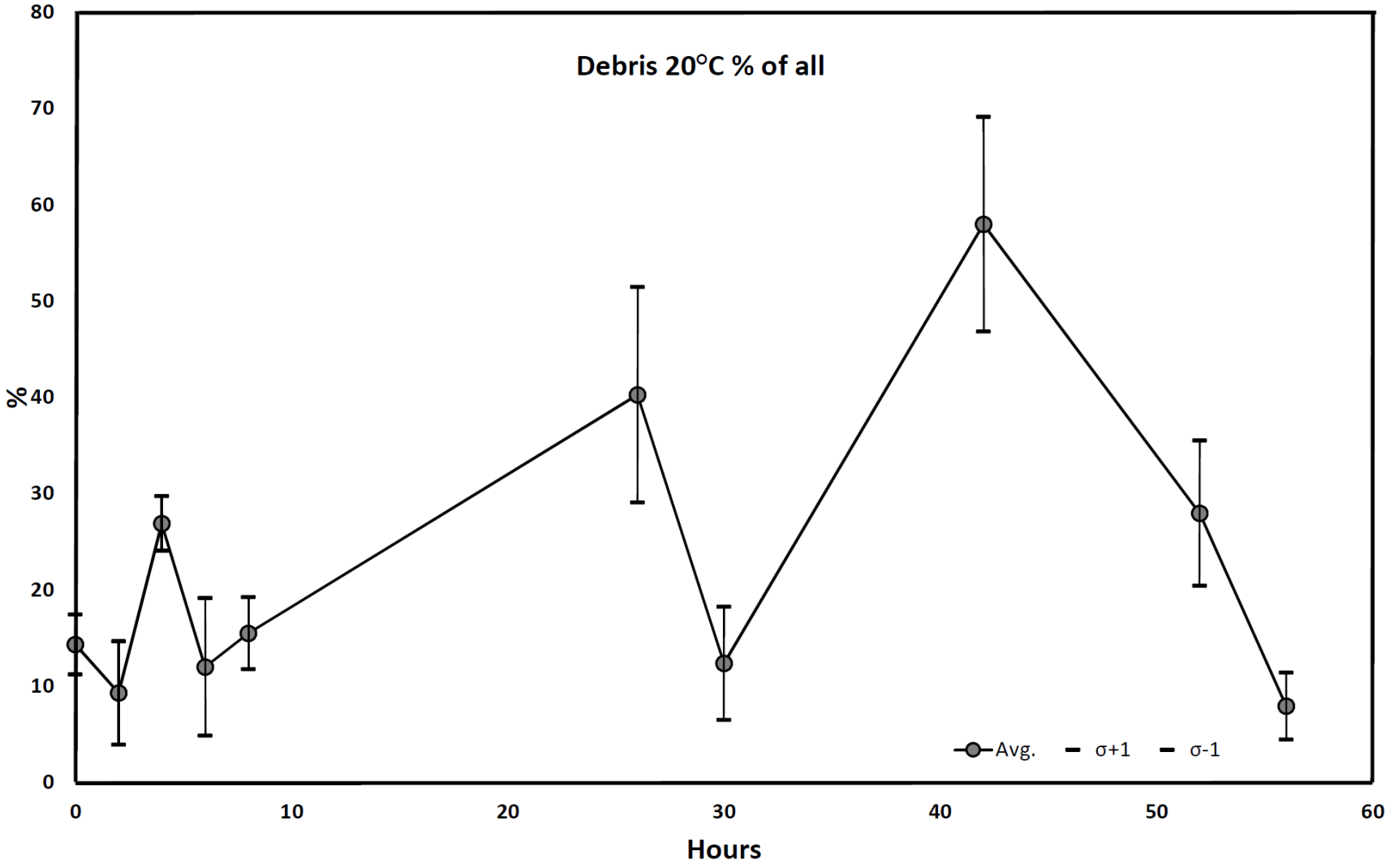


Figure 149. Chart of averages and standard deviation for cellular debris at 20 °C for all.

Appendix D17

26°C G1 Hours	% of P1 Avg.	Std Dev	σ+1	σ-1
0	39	6	45	33
2	41	3	44	38
4	56	7	63	49
6	58	6	64	53
8	34	6	40	29
26	22	6	28	16
30	29	7	36	22
42	35	4	39	30
52	37	8	44	29
56	43	5	49	38

Figure 150. Table of averages and standard deviation for G1 phase at 26 °C for P1.

Appendix D18

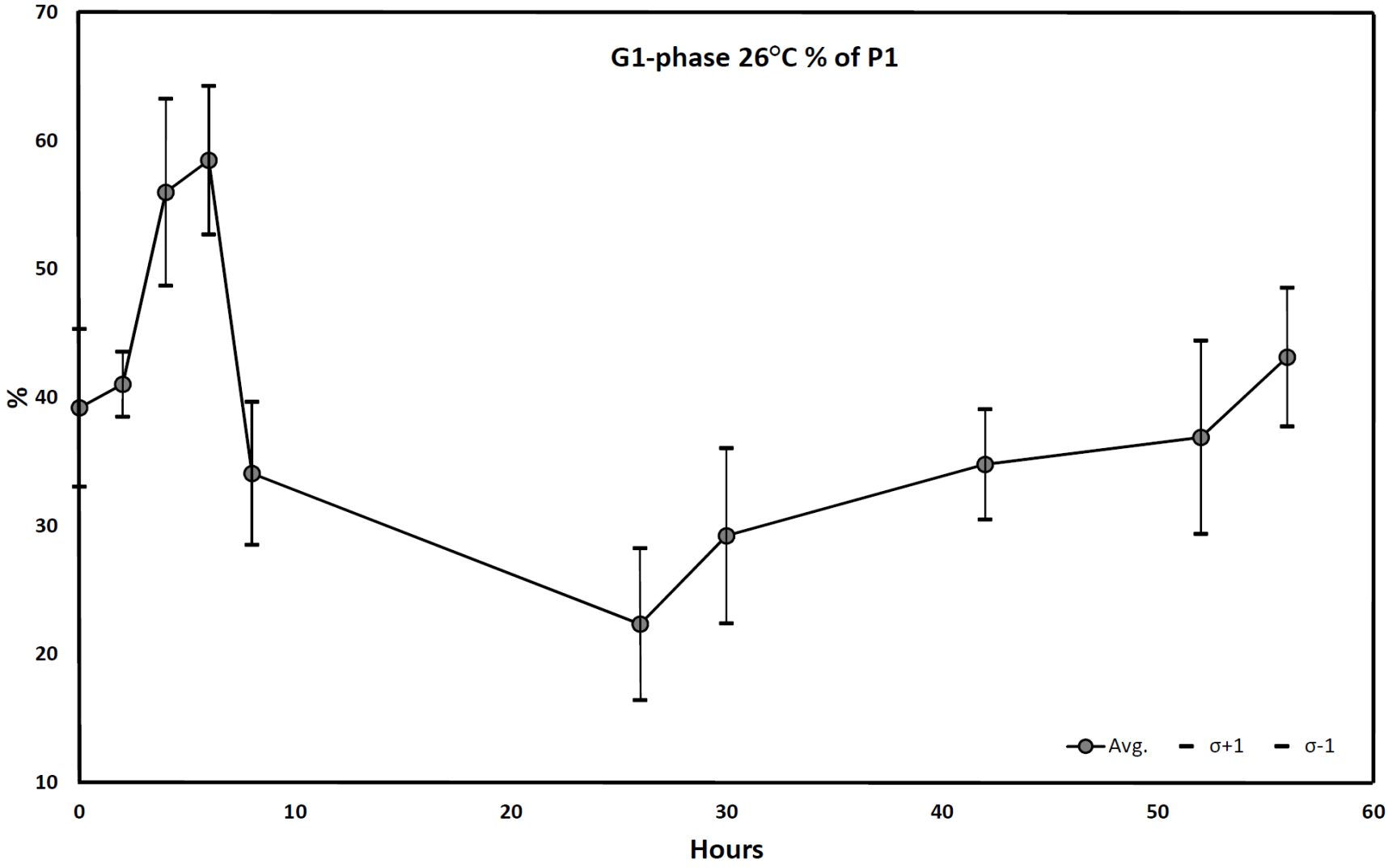


Figure 151. Chart of averages and standard deviation for G1 phase at 26 °C for P1.

Appendix D19

26°C S Hours	% of P1 Avg.	Std Dev	σ+1	σ-1
0	16	1	16	15
2	15	2	18	13
4	7	0	7	6
6	6	1	6	5
8	4	1	5	4
26	24	4	29	20
30	12	0	12	11
42	8	1	9	7
52	6	1	7	6
56	10	1	11	9

Figure 152. Table of averages and standard deviation for S phase at 26 °C for P1.

Appendix D20

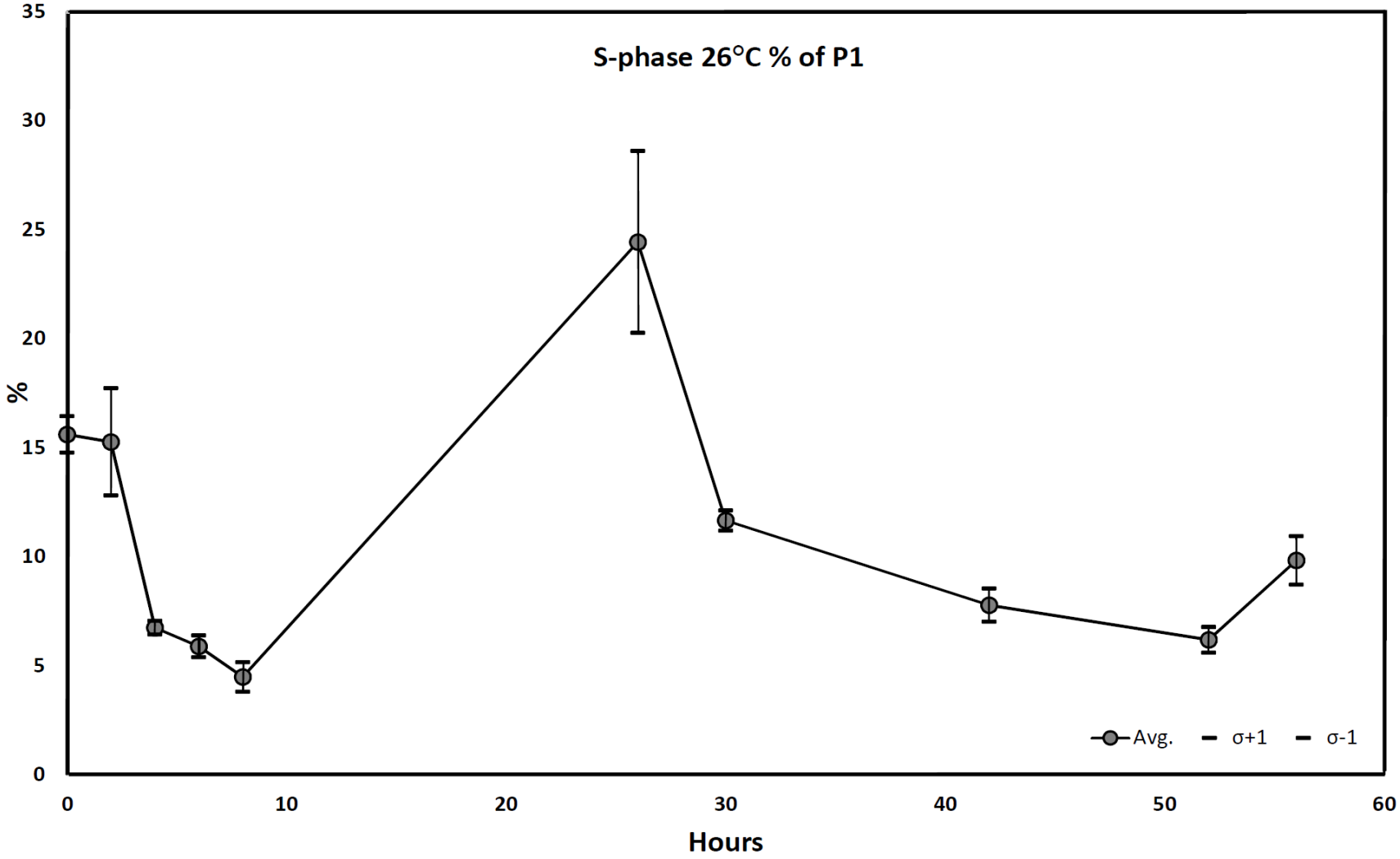


Figure 153. Chart of averages and standard deviation for S phase at 26 °C for P1.

Appendix D21

26°C G2/M Hours	% of P1 Avg.	Std Dev	σ+1	σ-1
0	31	3	34	28
2	29	4	33	25
4	27	6	33	21
6	28	4	32	24
8	42	1	43	42
26	29	5	33	24
30	38	1	39	37
42	37	3	40	34
52	39	3	42	37
56	28	3	31	26

Figure 154. Table of averages and standard deviation for G2/M phase at 26 °C for P1.

Appendix D22

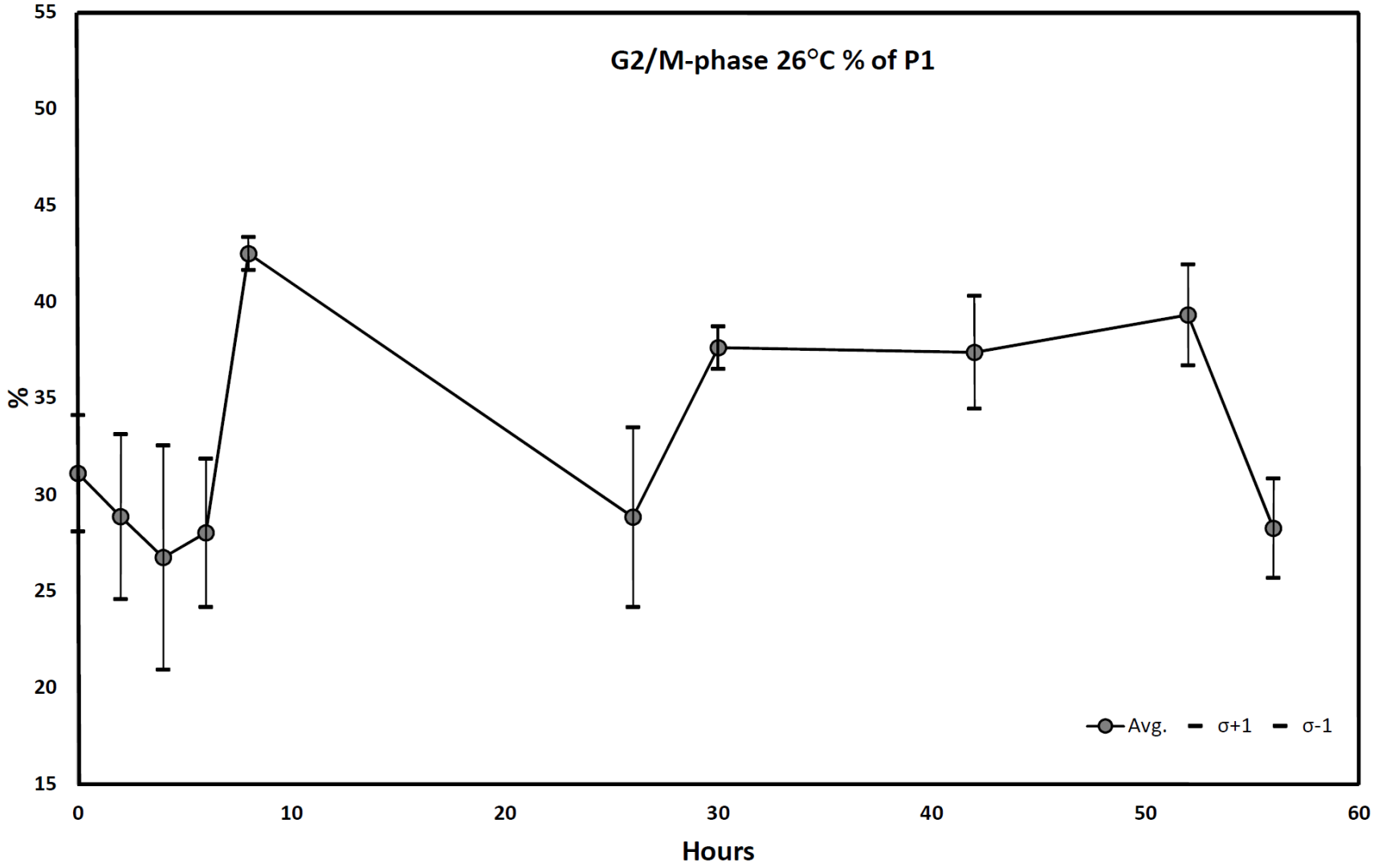


Figure 155. Chart of averages and standard deviation for G2/M phase at 26 °C for P1.

Appendix D23

26°C G1 Hours	% of all Avg.	Std Dev	σ+1	σ-1
0	28	5	34	23
2	35	1	36	34
4	22	7	29	15
6	47	4	51	43
8	24	5	29	18
26	9	4	13	5
30	21	2	23	18
42	16	6	22	10
52	21	3	24	19
56	34	4	39	30

Figure 156. Table of averages and standard deviation for G1 phase at 26 °C for all.

Appendix D24

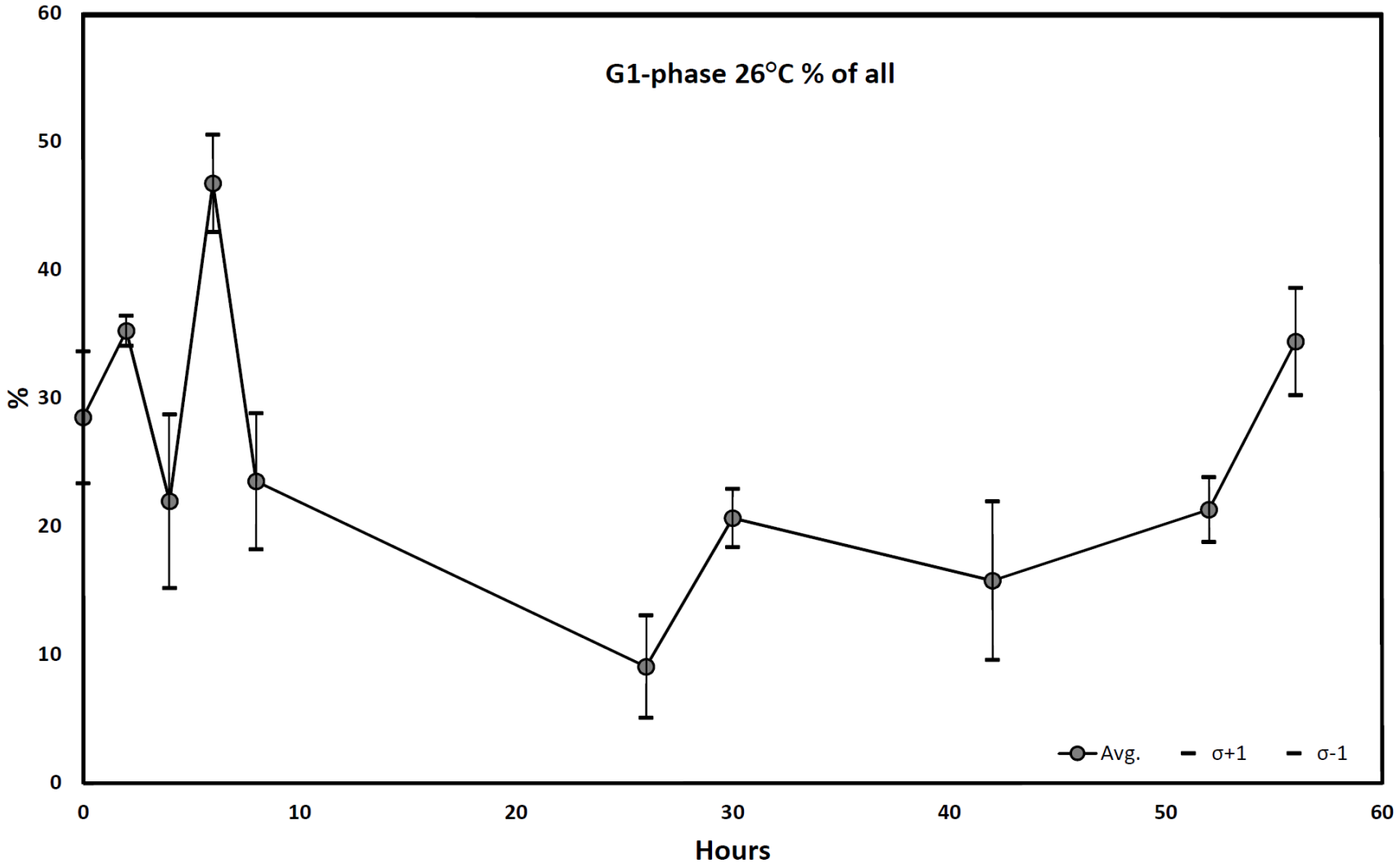


Figure 157. Chart of averages and standard deviation for G1 phase at 26 °C for all.

Appendix D25

26°C S Hours	% of all Avg.	Std Dev	σ+1	σ-1
0	12	3	14	9
2	13	1	14	12
4	3	1	3	2
6	5	1	6	4
8	3	0	3	3
26	10	4	14	6
30	8	1	10	7
42	4	1	5	2
52	4	1	4	3
56	8	1	8	7

Figure 158. Table of averages and standard deviation for S phase at 26 °C for all.

Appendix D26

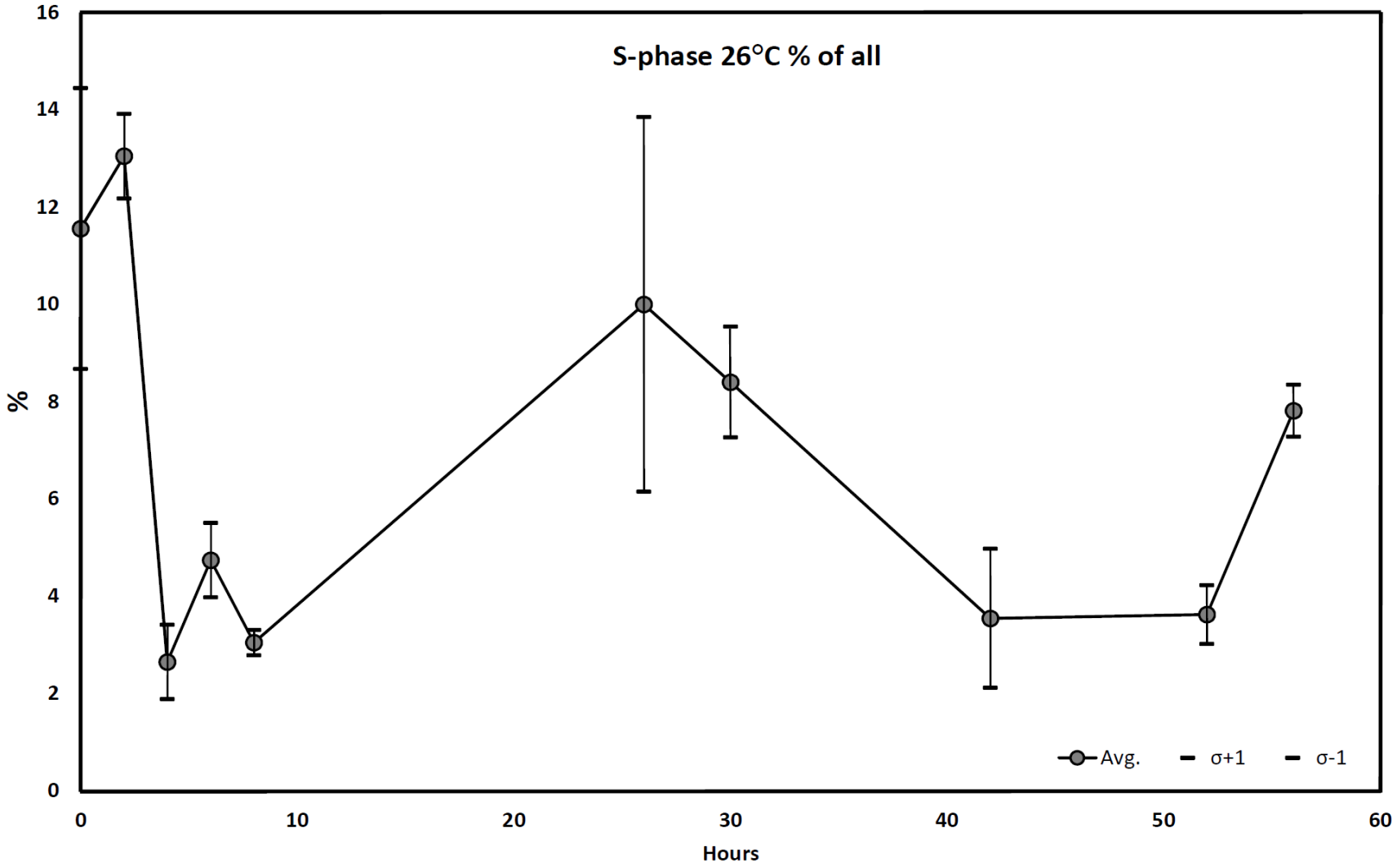


Figure 159. Chart of averages and standard deviation for S phase at 26 °C for all.

Appendix D27

26°C G2/M Hours	% of all Avg.	Std Dev	σ+1	σ-1
0	24	9	32	15
2	25	6	31	19
4	11	4	15	6
6	23	5	28	18
8	29	2	31	27
26	13	7	21	6
30	27	5	32	22
42	18	8	26	9
52	24	7	31	16
56	23	4	27	19

Figure 160. Table of averages and standard deviation for G2/M phase at 26 °C for all.

Appendix D28

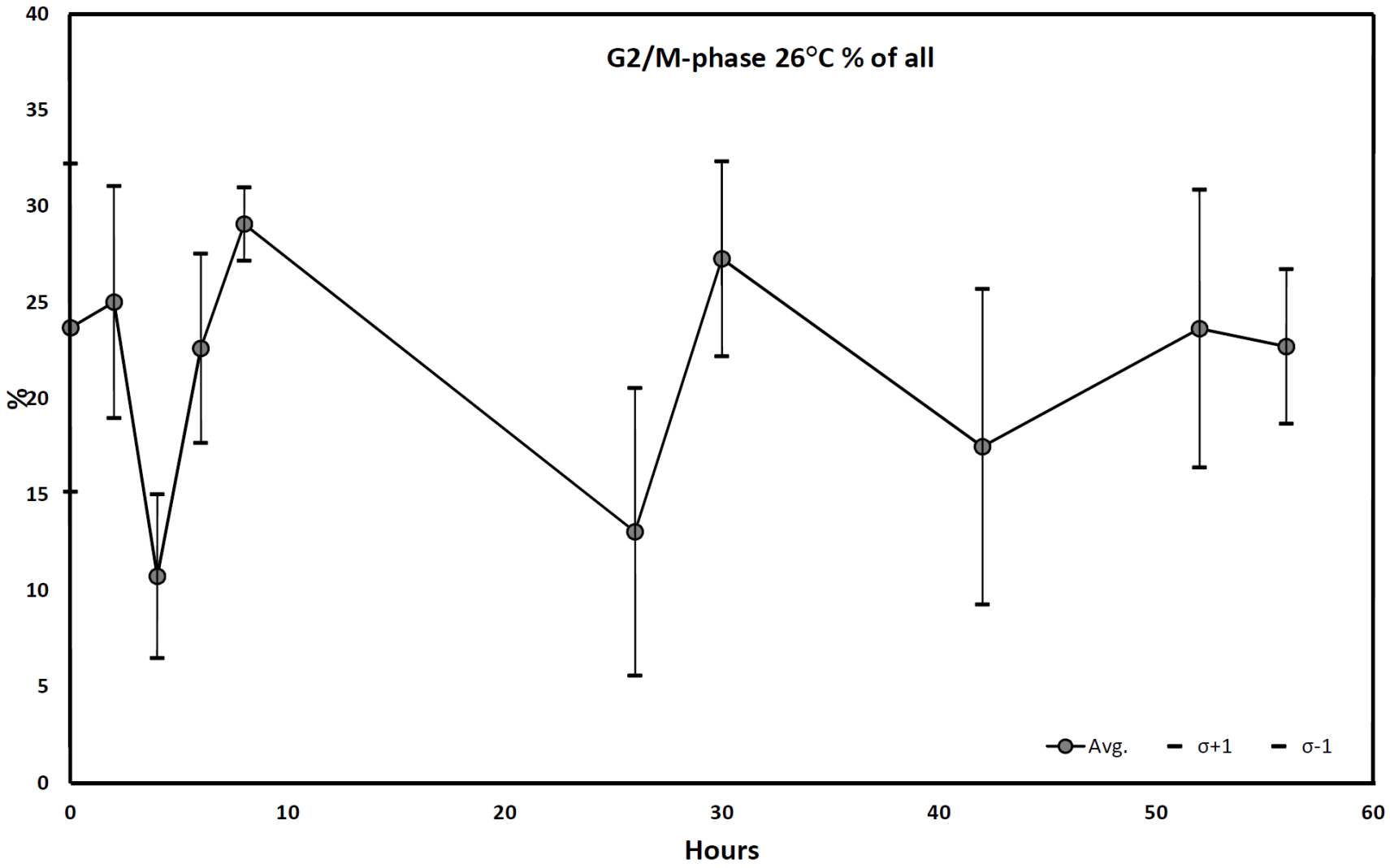


Figure 161. Chart of averages and standard deviation for G2/M phase at 26 °C for all.

Appendix D29

26°C Debris Hours	% of all Avg.	Std Dev	σ+1	σ-1
0	21	18	39	2
2	11	7	18	4
4	52	10	62	41
6	16	8	24	8
8	26	4	30	22
26	48	20	68	28
30	23	10	33	12
42	47	19	65	28
52	34	13	46	21
56	15	6	21	9

Figure 162. Table of averages and standard deviation for cellular debris at 26 °C for all.

Appendix D30

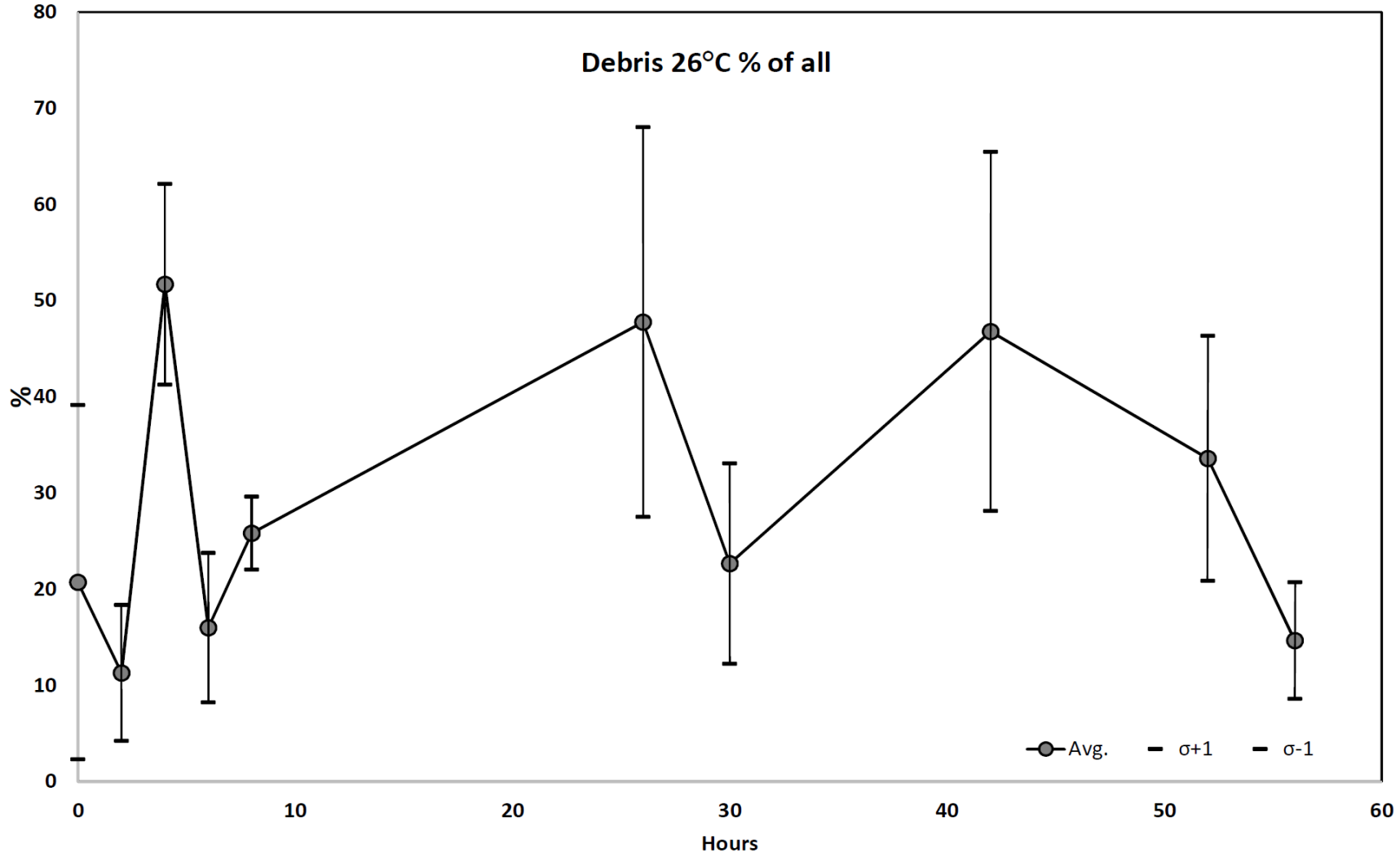


Figure 163. Chart of averages and standard deviation for cellular debris at 26 °C for all.

Appendix D31

G1	% of P1		
Hours	20°C	26°C	Dif.
0	36	39	3
2	38	41	4
4	55	56	1
6	51	58	7
8	34	34	0
26	44	22	-22
30	35	29	-5
42	42	35	-7
52	37	37	0
56	45	43	-2

Figure 164. Table comparing G1 phase at 20 °C and 26 °C for P1. The table also notes the difference between 20 °C and 26 °C.

Appendix D32

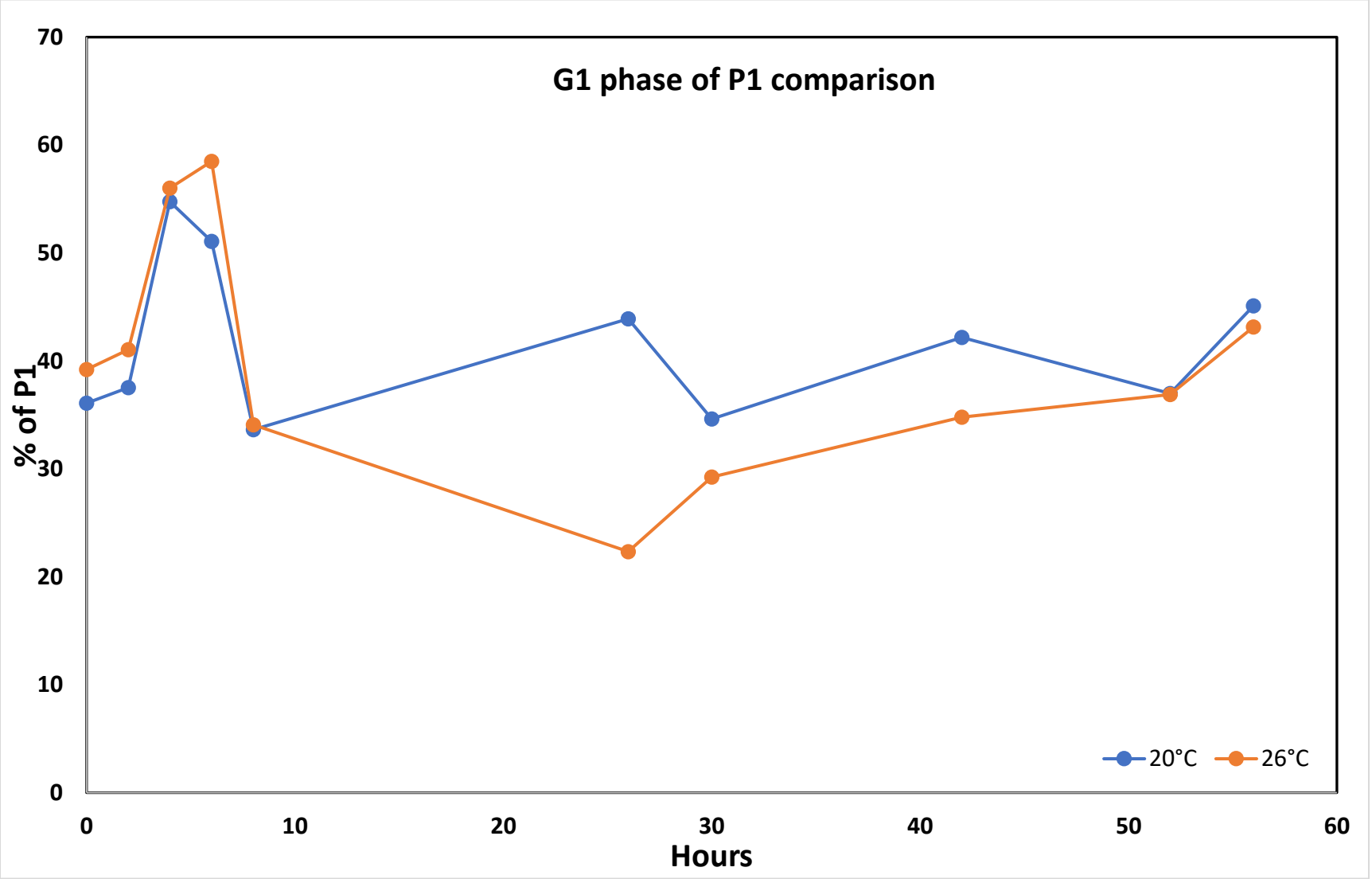


Figure 165. Chart comparing G1 phase at 20 °C and 26 °C for P1.

Appendix D33

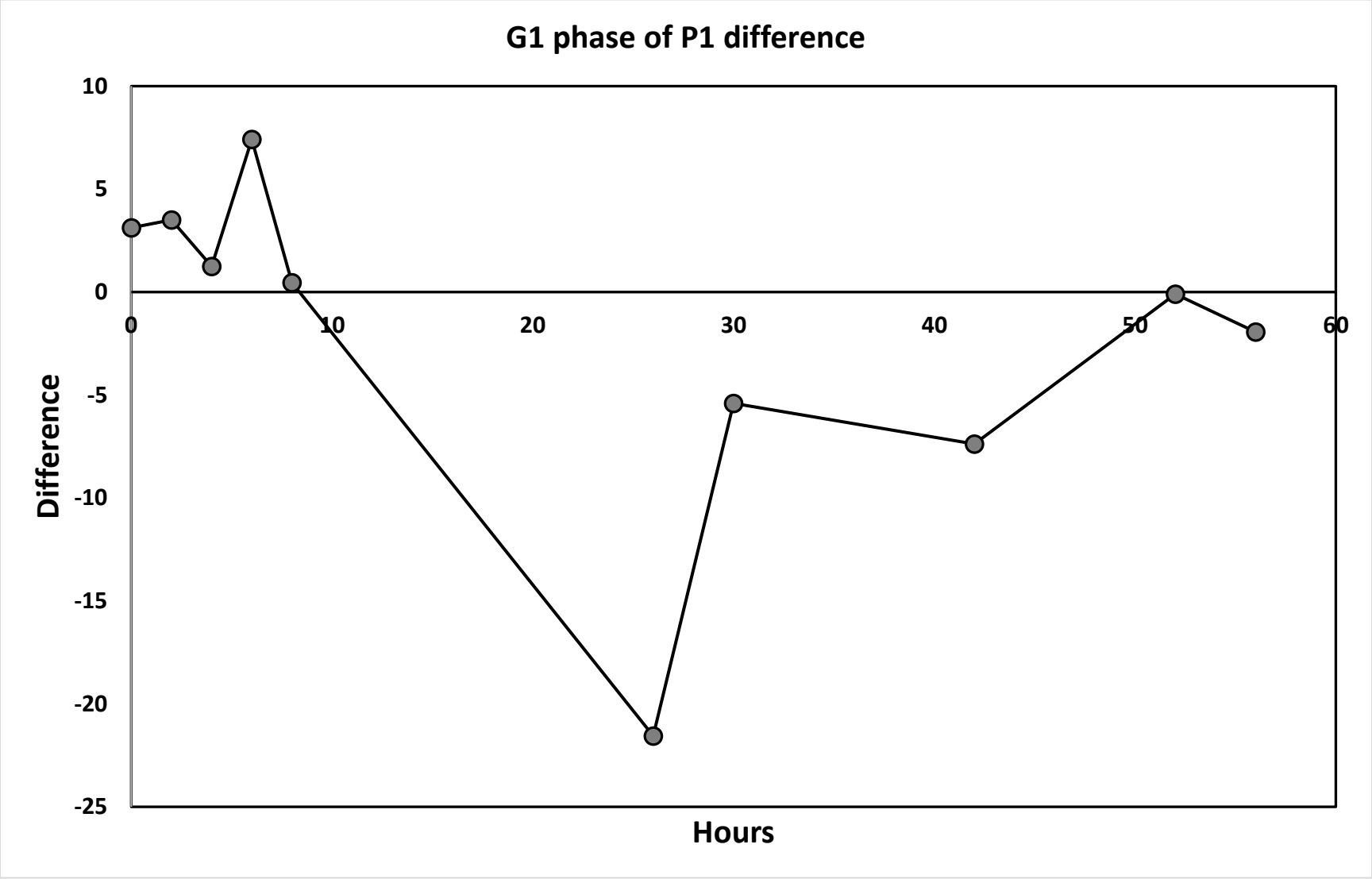


Figure 166. Chart showing the difference between 20 °C and 26 °C for G1 phase of P1.

Appendix D34

S	% P1		
Hours	20°C	26°C	Dif.
0	15	16	1
2	11	15	4
4	12	7	-5
6	9	6	-3
8	4	4	0
26	13	24	12
30	6	12	6
42	14	8	-6
52	8	6	-2
56	10	10	0

Figure 167. Table comparing S phase at 20 °C and 26 °C for P1. The table also notes the difference between 20 °C and 26 °C.

Appendix D35

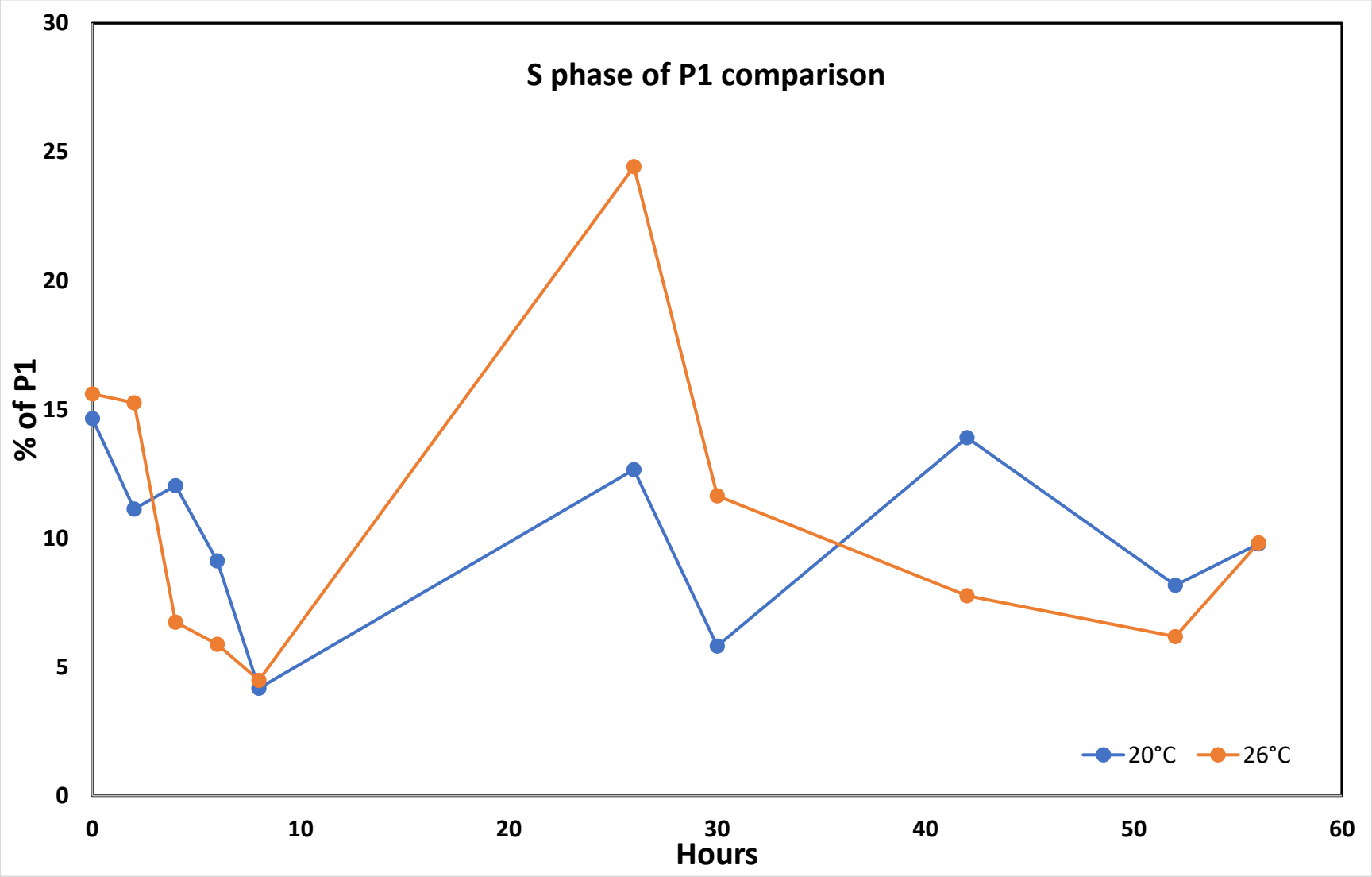


Figure 168. Chart comparing S phase at 20 °C and 26 °C for P1.

Appendix D36

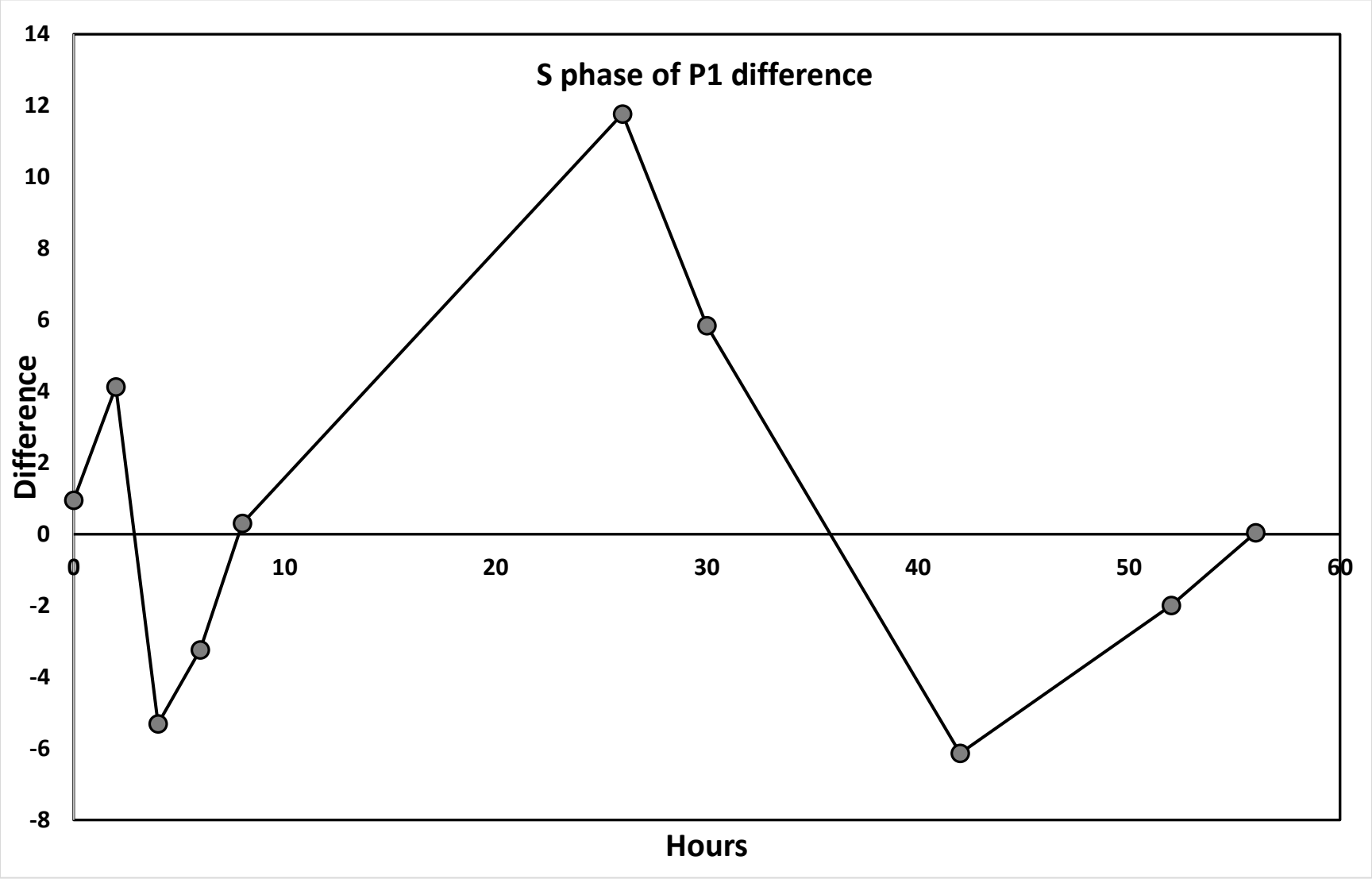


Figure 169. Chart showing the difference between 20 °C and 26 °C for S phase of P1.

Appendix D37

G2/M	% P1		
Hours	20°C	26°C	Dif.
0	35	31	-4
2	34	29	-5
4	26	27	1
6	28	28	0
8	41	42	1
26	29	29	0
30	39	38	-2
42	28	37	9
52	33	39	6
56	27	28	1

Figure 170. Table comparing G2/M phase at 20 °C and 26 °C for P1. The table also notes the difference between 20 °C and 26 °C.

Appendix D38

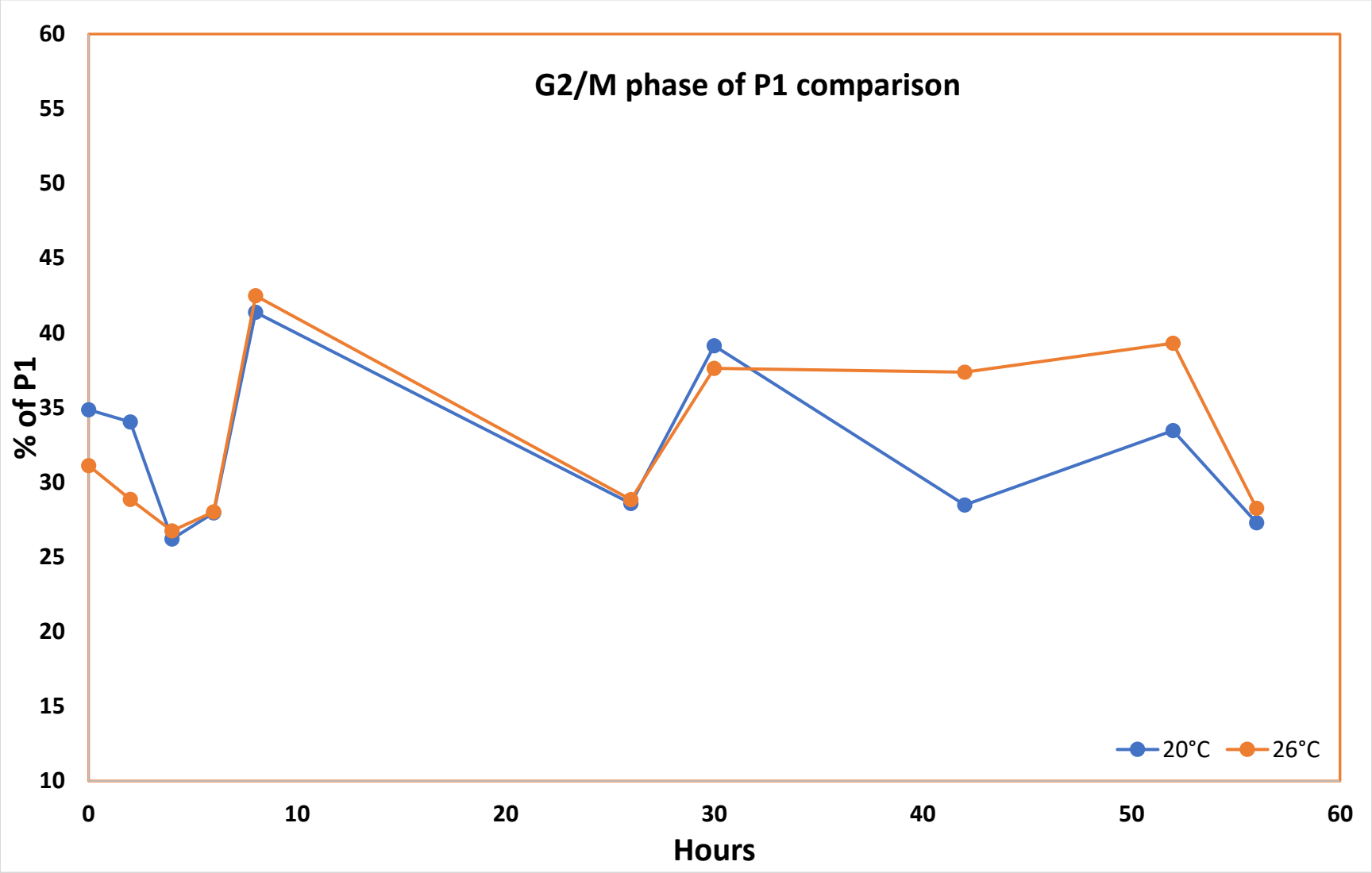


Figure 171. Chart comparing G2/M phase at 2 0 °C and 26 °C for P1.

Appendix D39

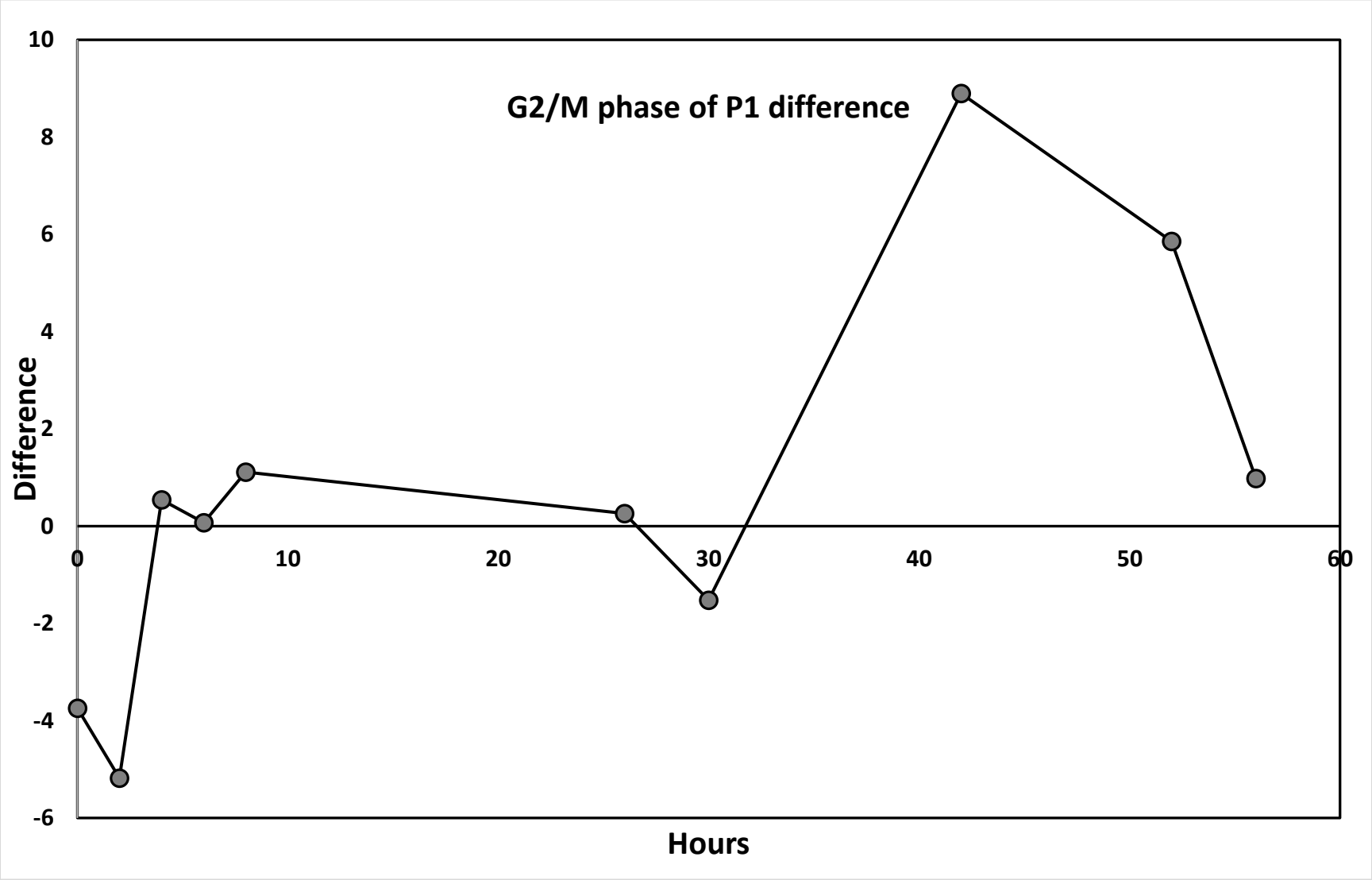


Figure 172. Chart showing the difference between 20 °C and 26 °C for G2/M phase of P1.

Appendix D40

G1	% all		
Hours	20°C	26°C	Dif.
0	30	28	-1
2	33	35	3
4	37	22	-15
6	43	47	4
8	27	24	-3
26	24	9	-15
30	29	21	-9
42	14	16	2
52	25	21	-3
56	40	34	-5

Figure 173. Table comparing G1 phase at 20 °C and 26 °C of all. The table also notes the difference between 20 °C and 26 °C.

Appendix D41

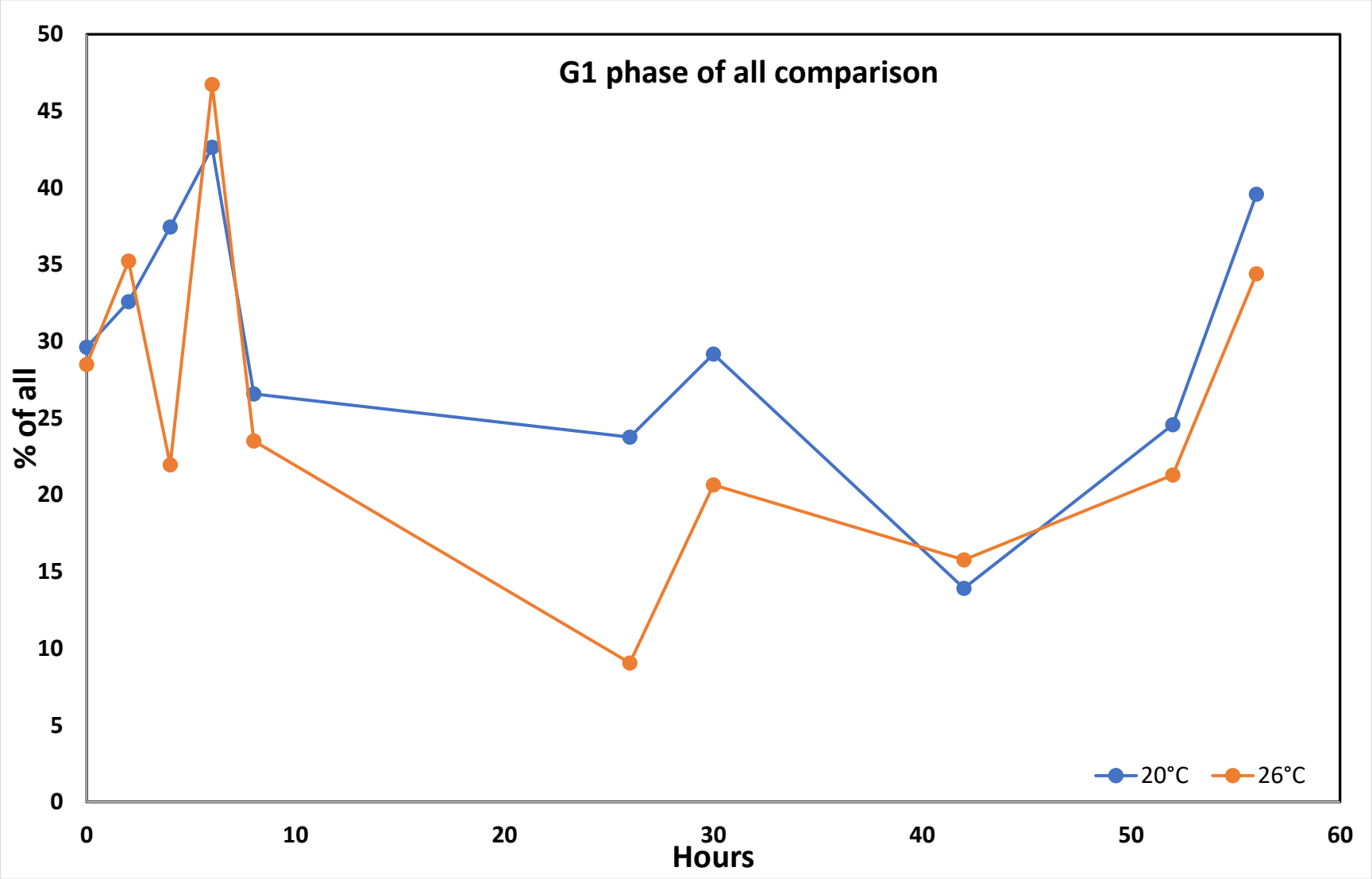


Figure 174. Chart comparing G1 phase at 20 °C and 26 °C of all.

Appendix D42

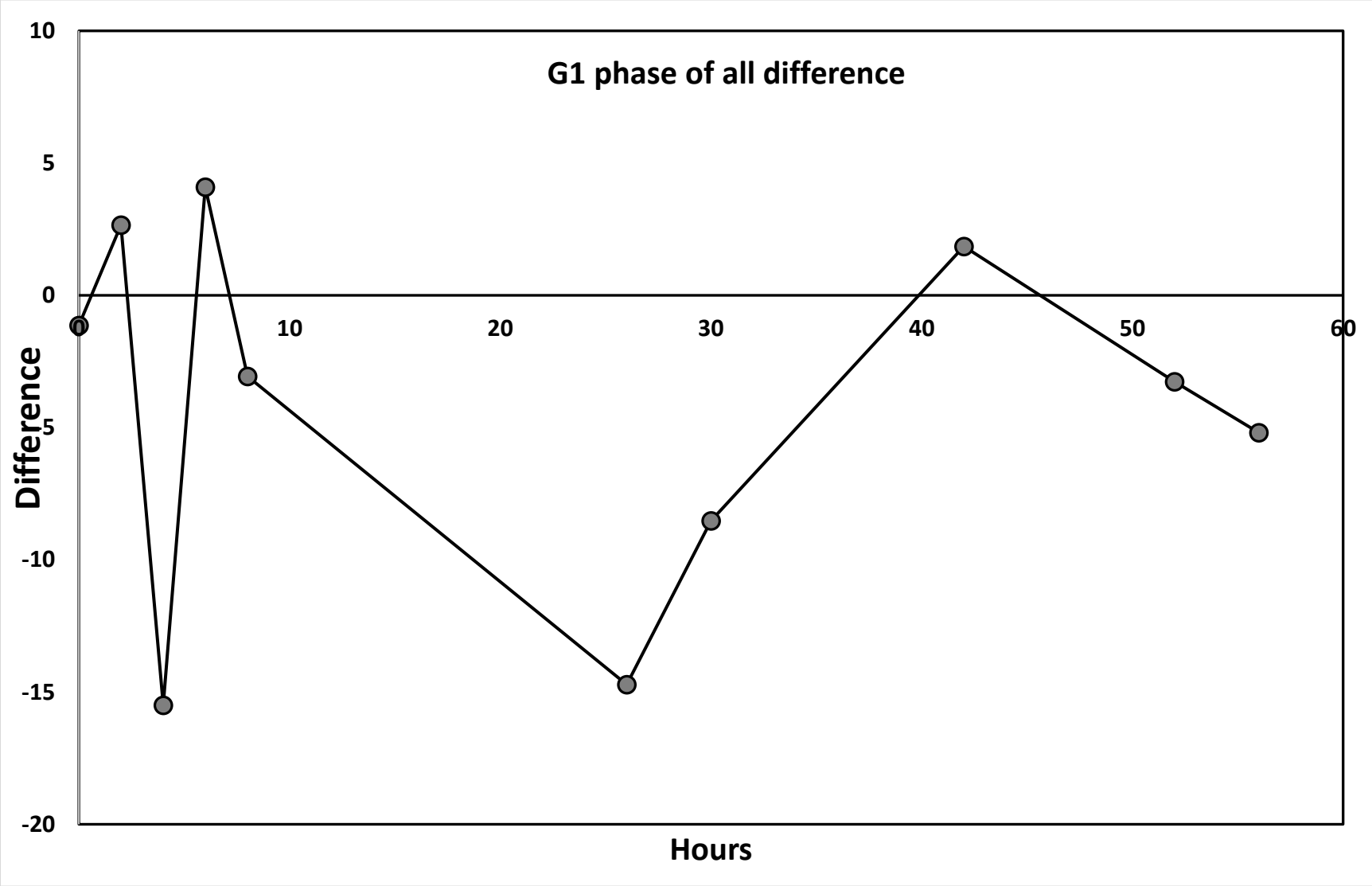


Figure 175. Chart showing the difference between 20 °C and 26 °C for G1 phase of all.

Appendix D43

S	% all		
Hours	20°C	26°C	Dif.
0	12	12	-1
2	10	13	3
4	8	3	-6
6	8	5	-3
8	3	3	0
26	7	10	3
30	5	8	4
42	5	4	-1
52	5	4	-2
56	9	8	-1

Figure 176. Table comparing S phase at 20 °C and 26 °C of all. The table also notes the difference between 20 °C and 26 °C.

Appendix D44

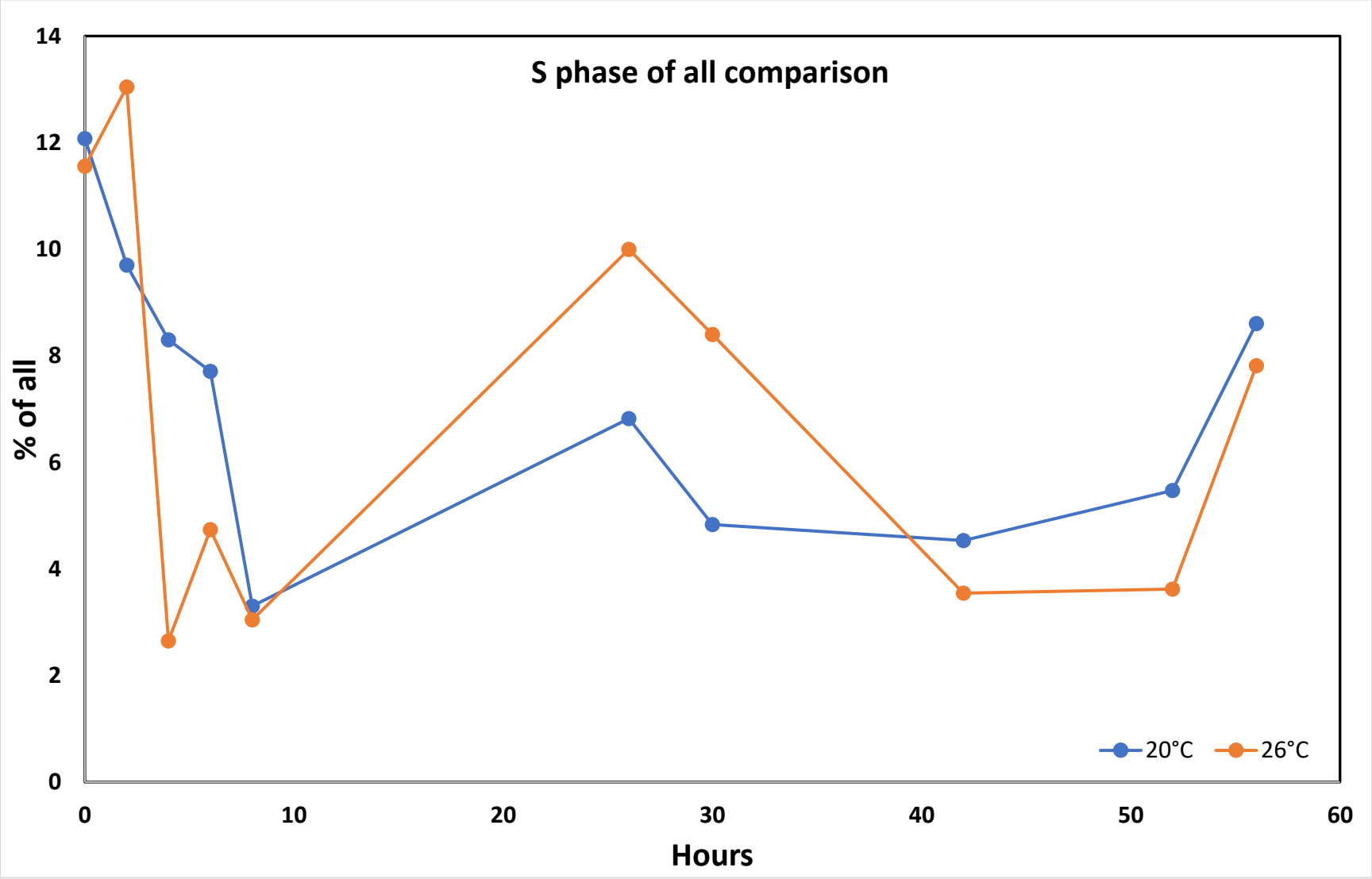


Figure 177. Chart comparing S phase at 20 °C and 26 °C of all.

Appendix D45

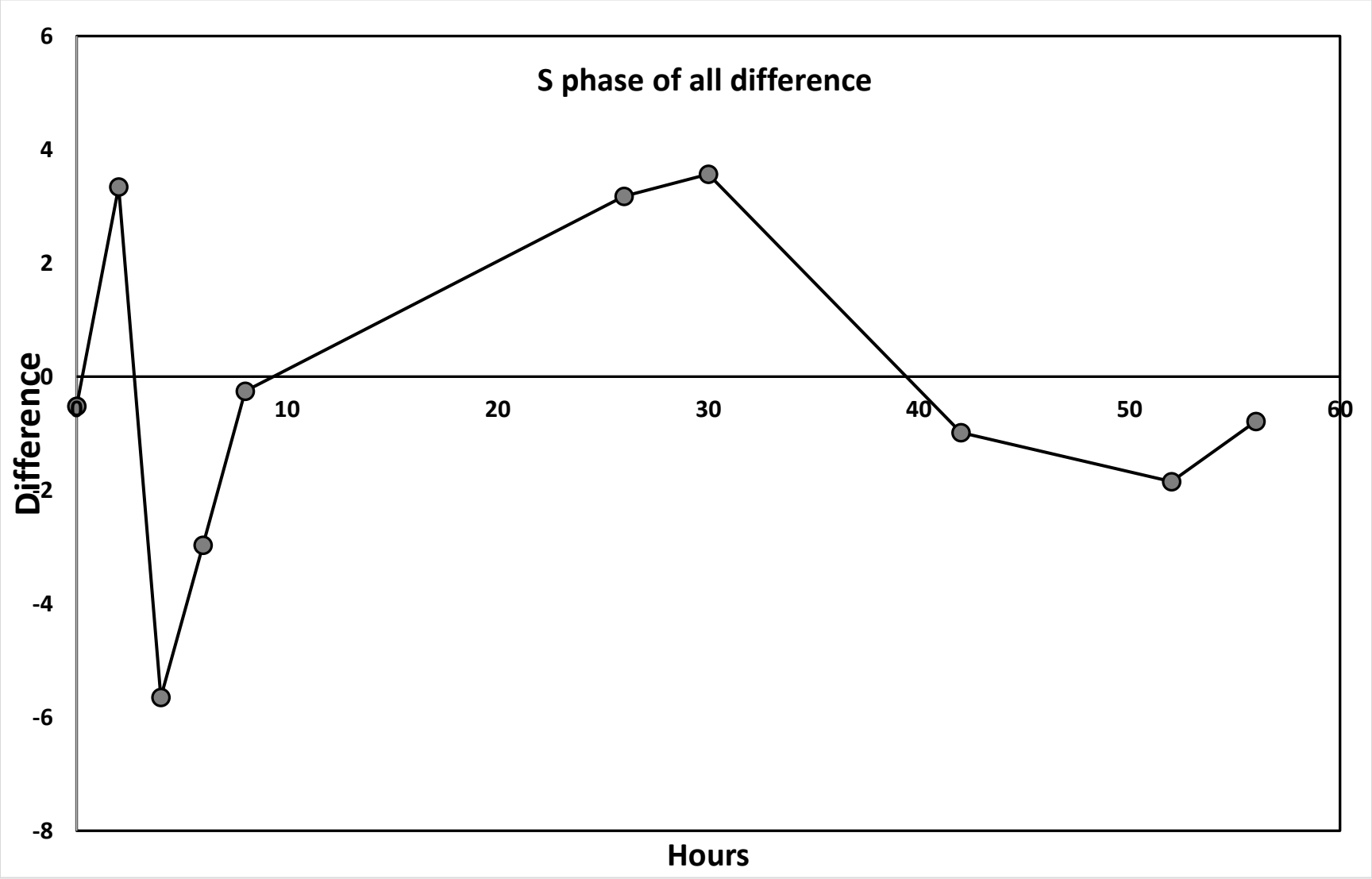


Figure 178. Chart showing the difference between 20 °C and 26 °C for S phase of all.

Appendix D46

G2/M	% all		
Hours	20°C	26°C	Dif.
0	27	24	-4
2	30	25	-5
4	23	11	-12
6	24	23	-1
8	33	29	-4
26	15	13	-2
30	33	27	-5
42	9	18	8
52	22	24	1
56	24	23	-1

Figure 179. Table comparing G2/M phase at 20 °C and 26 °C of all. The table also notes the difference between 20 °C and 26 °C.

Appendix D47

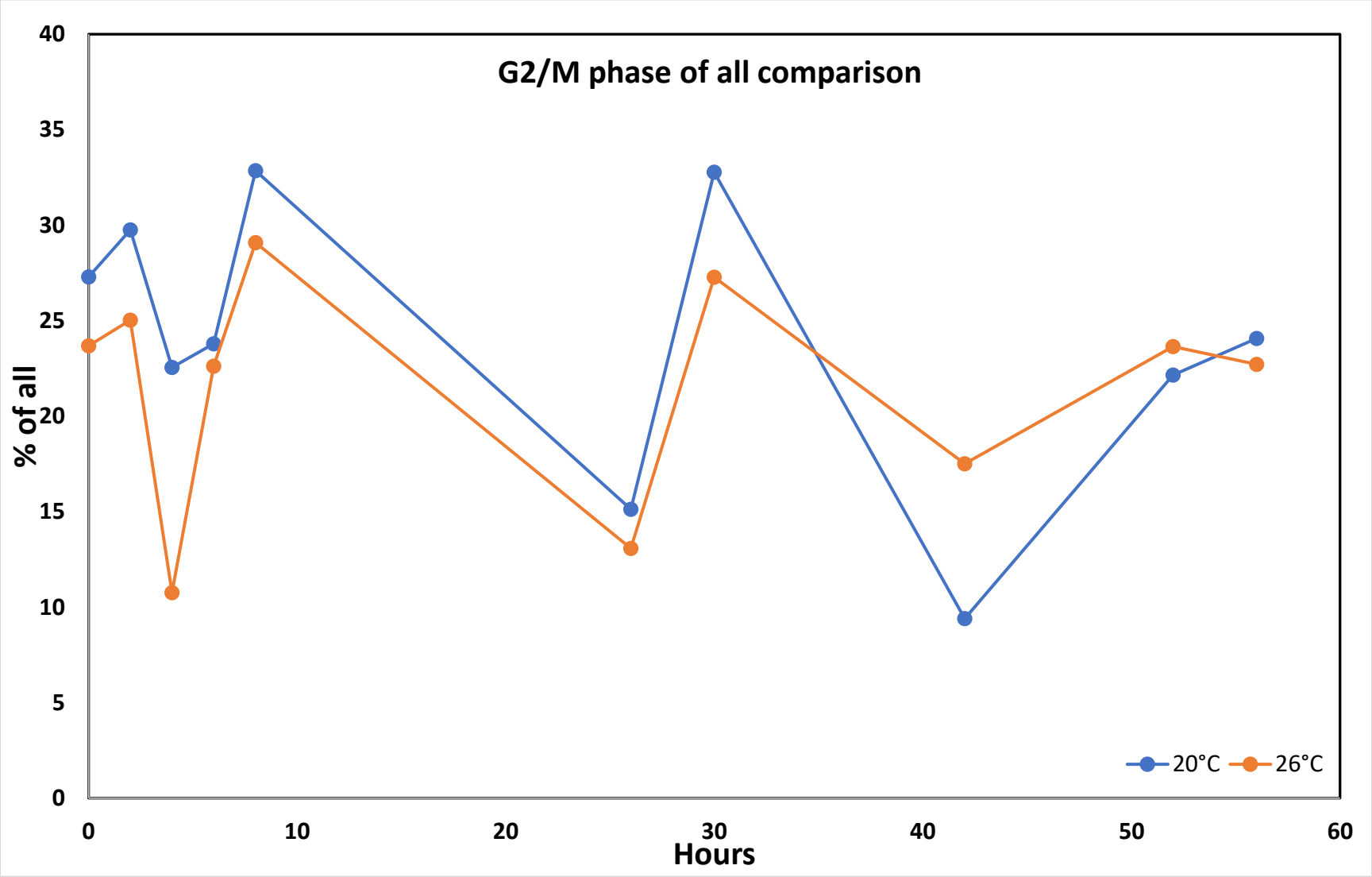


Figure 180. Chart comparing G2/M phase at 20 °C and 26 °C of all.

Appendix D48

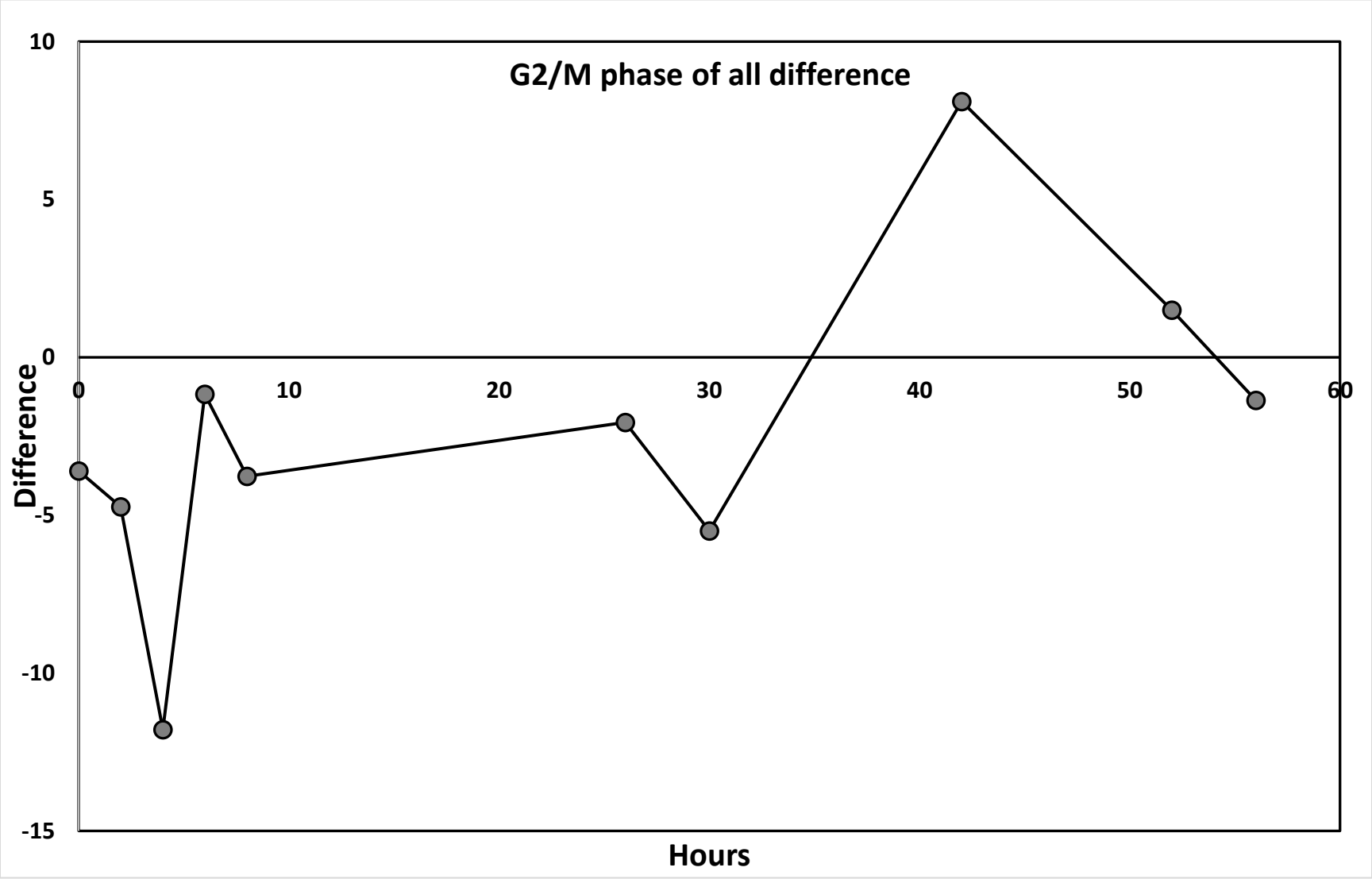


Figure 181. Chart showing the difference between 20 °C and 26 °C for G2/M phase of all.

Appendix D49

Debris	% all		
Hours	20°C	26°C	Dif.
0	14	21	6
2	9	11	2
4	27	52	25
6	12	16	4
8	16	26	10
26	40	48	8
30	12	23	10
42	58	47	-11
52	28	34	6
56	8	15	7

Figure 182. Table comparing Cellular debris at 20 °C and 26 °C of all. The table also notes the difference between 20 °C and 26 °C.

Appendix D50

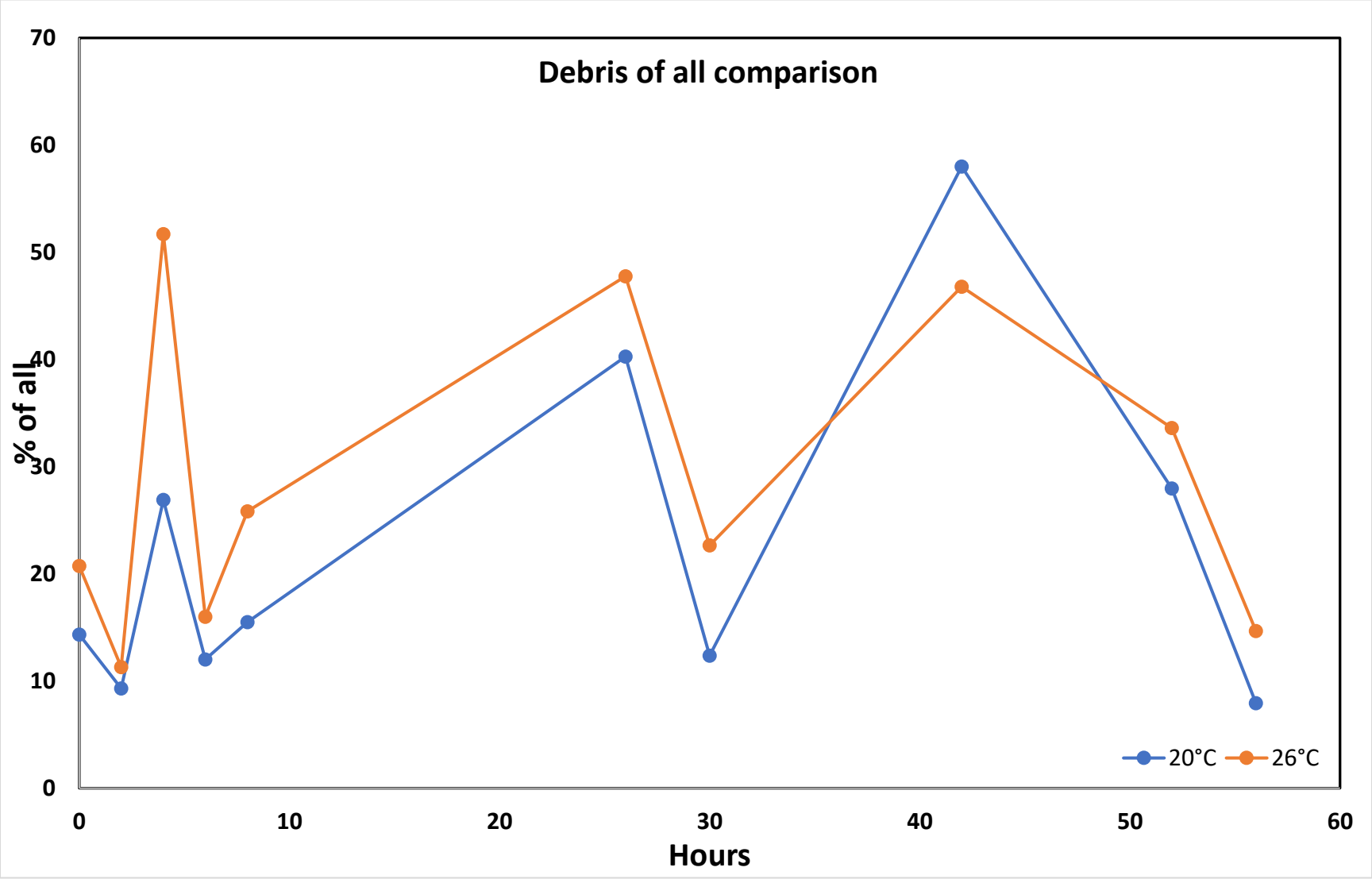


Figure 183. Chart comparing Cellular debris at 20 °C and 26 °C of all.

Appendix D51

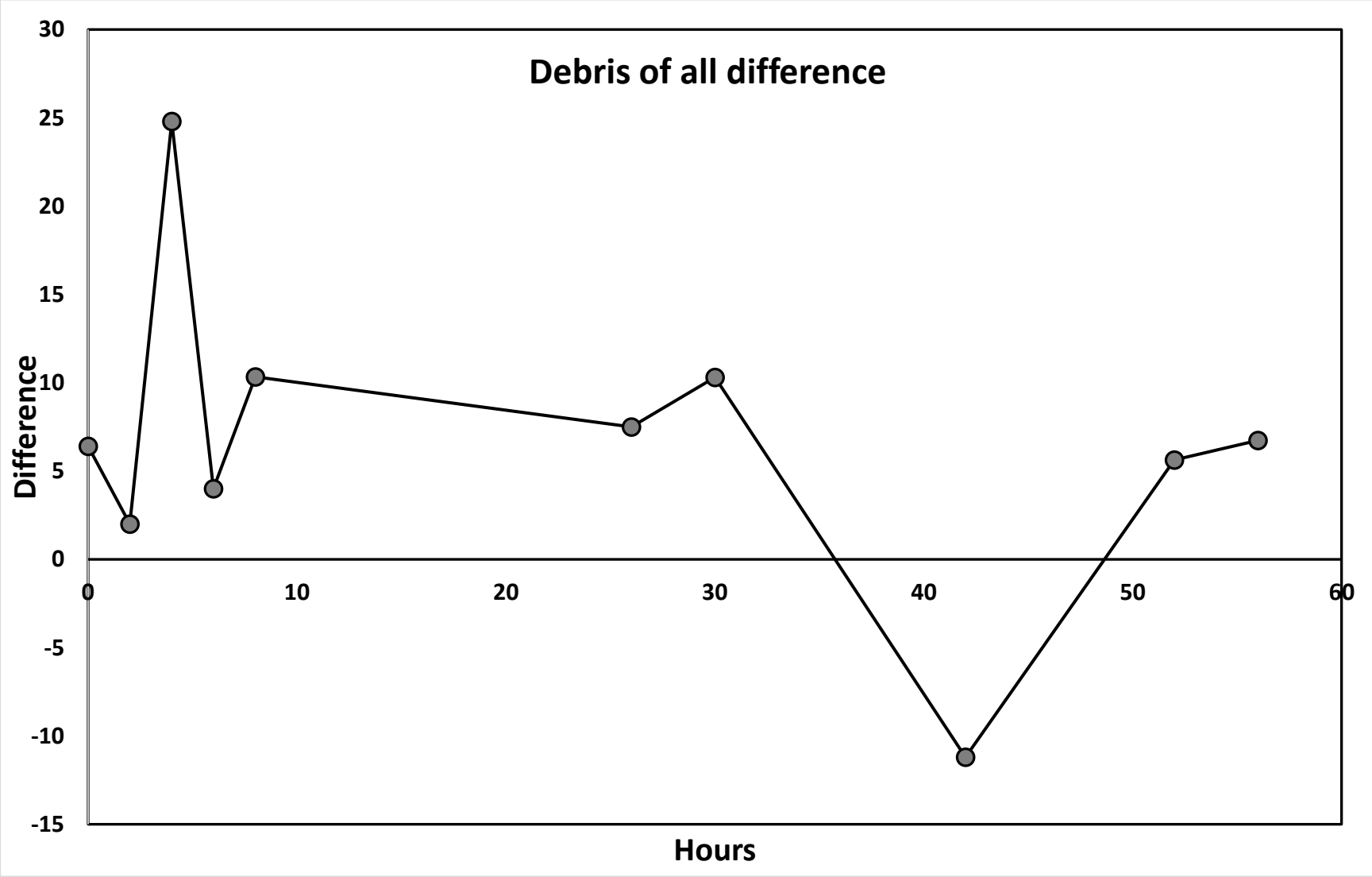


Figure 184. Chart showing the difference between 20 °C and 26 °C for cellular debris of all.

Appendix D52

20°C	% P1		
Hours	G1	S	G2/M
0	36	15	35
2	38	11	34
4	55	12	26
6	51	9	28
8	34	4	41
26	44	13	29
30	35	6	39
42	42	14	28
52	37	8	33
56	45	10	27

Figure 185. Table of cell cycle at 20 °C of P1.

Appendix D53

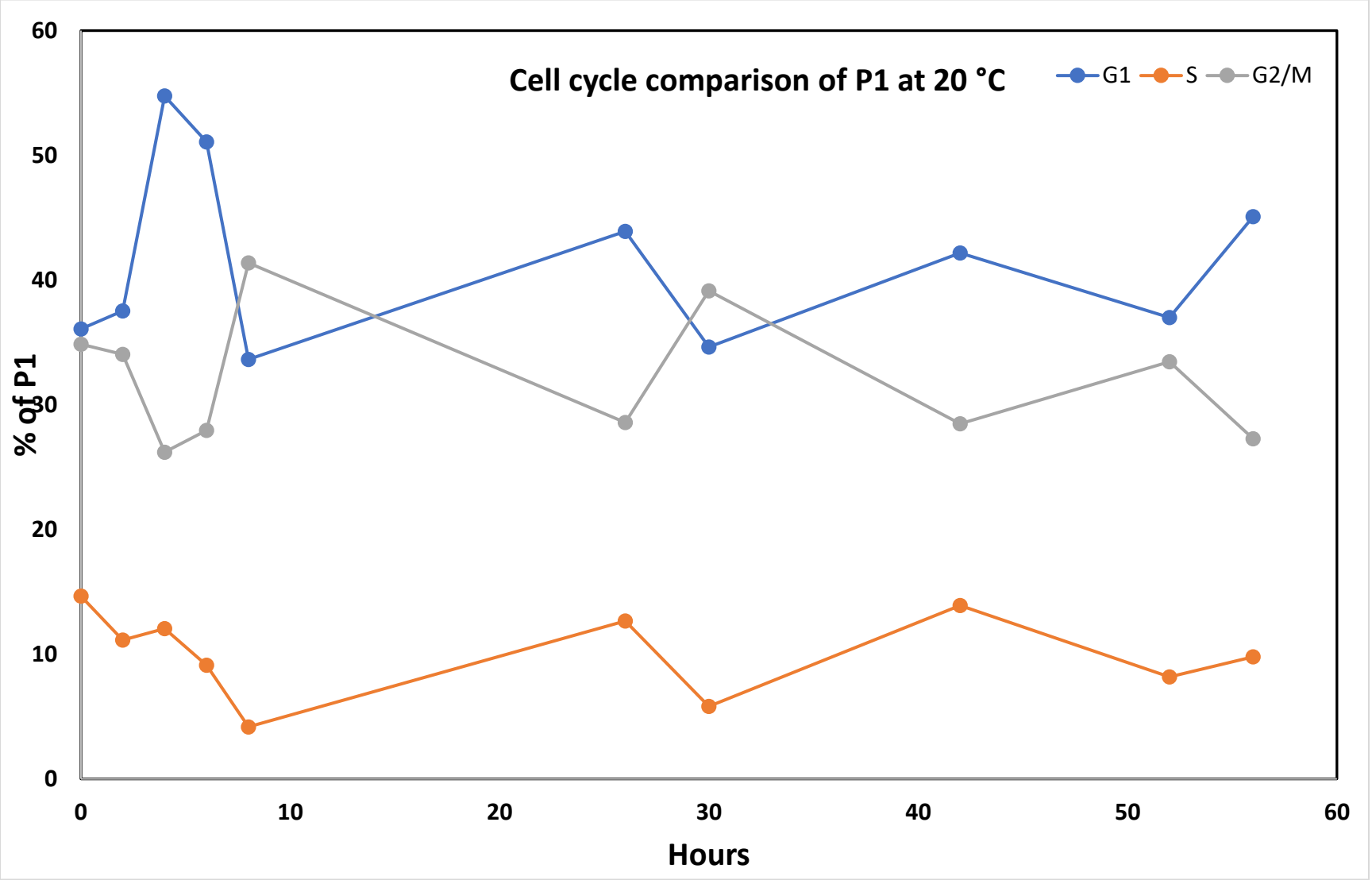


Figure 186. Chart of cell cycle at 20 °C of all.

Appendix D54

26°C	% P1		
Hours	G1	S	G2/M
0	39	16	31
2	41	15	29
4	56	7	27
6	58	6	28
8	34	4	42
26	22	24	29
30	29	12	38
42	35	8	37
52	37	6	39
56	43	10	28

Figure 187. Table of cell cycle at 26 °C of P1.

Appendix D55

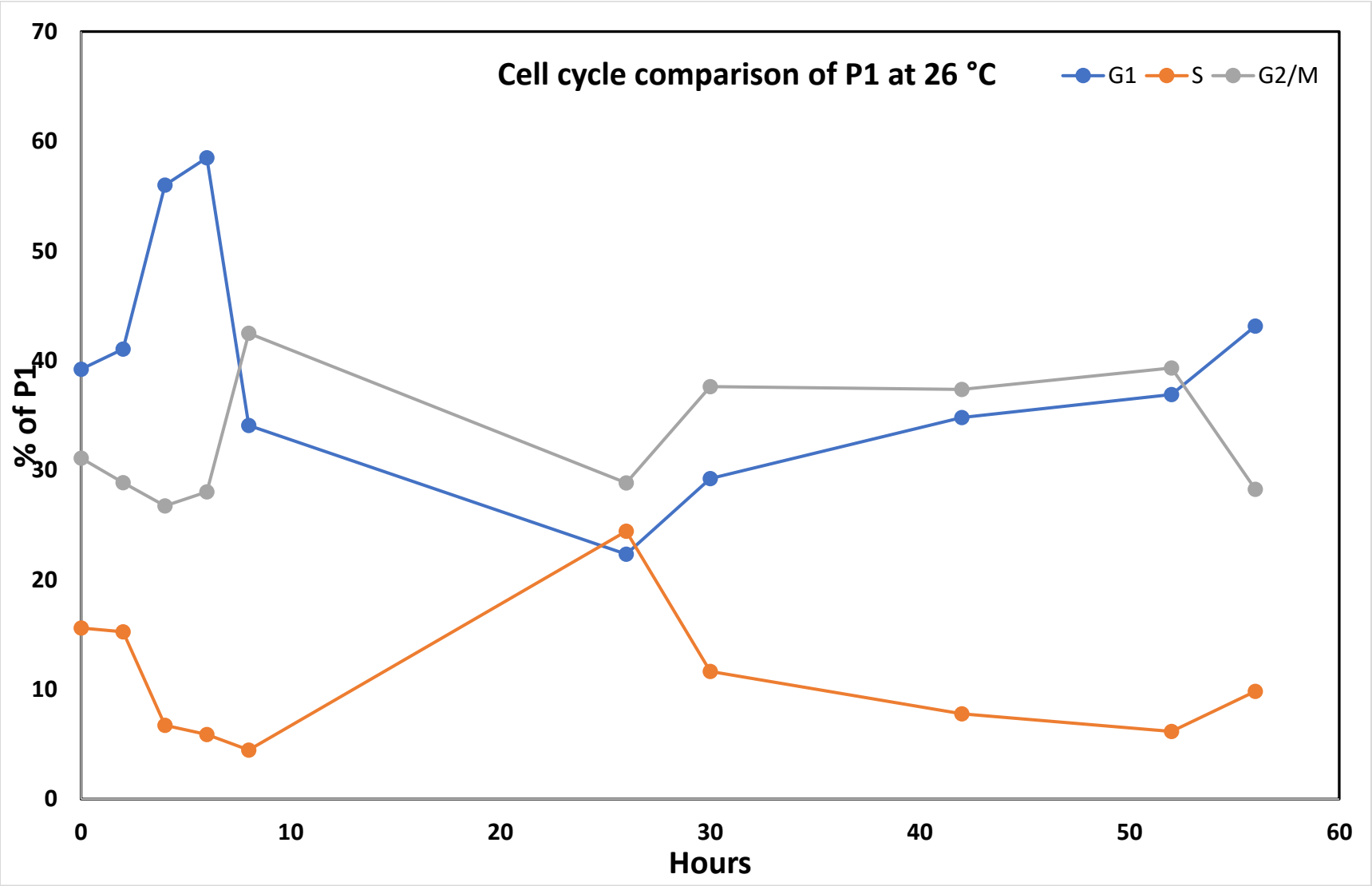


Figure 188. Chart of cell cycle at 26 °C of P1.

Appendix D56

20°C	% all			
Hours	G1	S	G2/M	Debris
0	30	12	27	14
2	33	10	30	9
4	37	8	23	27
6	43	8	24	12
8	27	3	33	16
26	24	7	15	40
30	29	5	33	12
42	14	5	9	58
52	25	5	22	28
56	40	9	24	8

Figure 189. Table of cell cycle at 20 °C of all.

Appendix D57

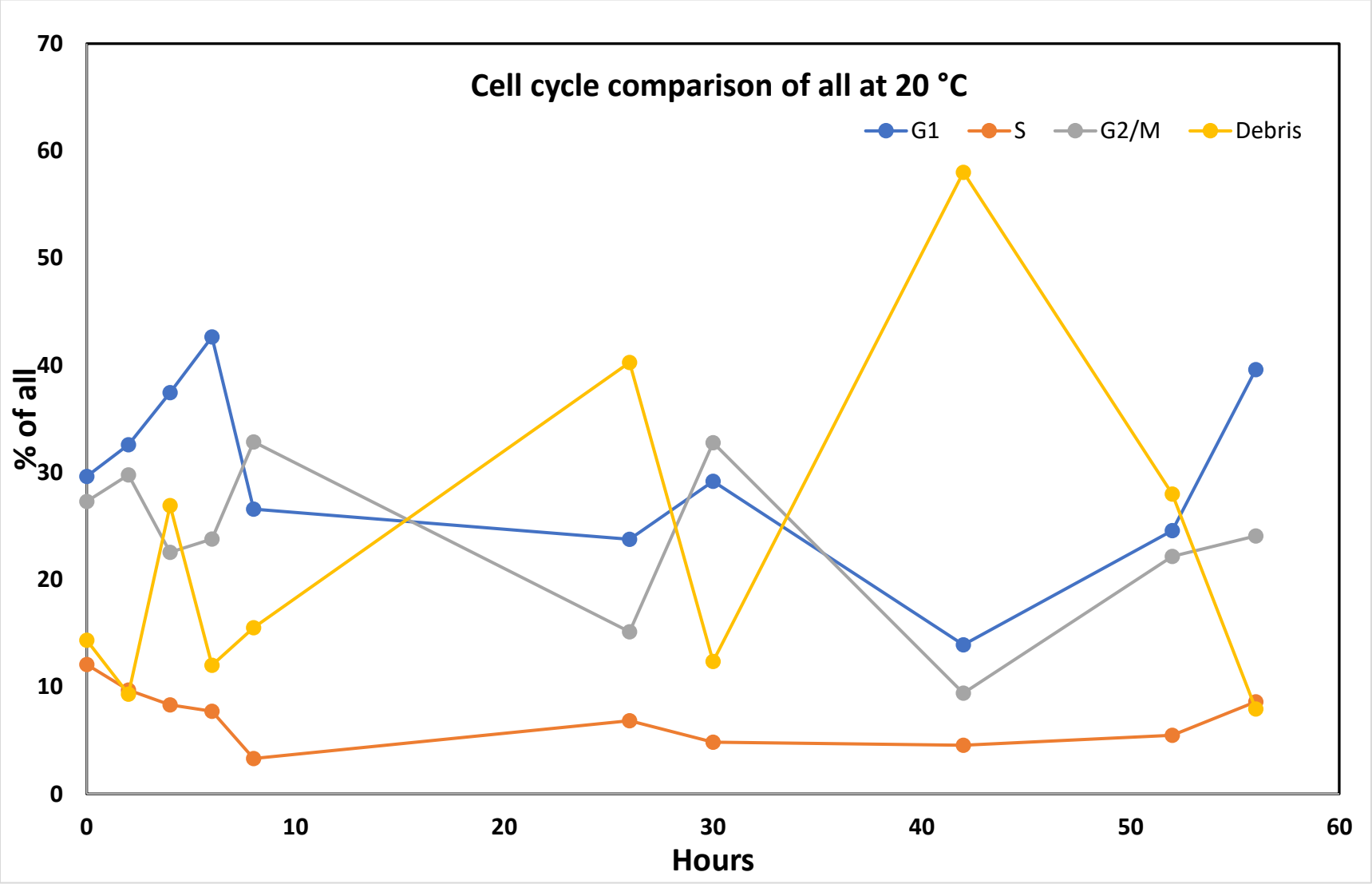


Figure 190. Chart of cell cycle at 20 °C of all.

Appendix D58

20°C	% all			
Hours	G1	S	G2/M	Debris
0	28	12	24	21
2	35	13	25	11
4	22	3	11	52
6	47	5	23	16
8	24	3	29	26
26	9	10	13	48
30	21	8	27	23
42	16	4	18	47
52	21	4	24	34
56	34	8	23	15

Figure 191. Table of cell cycle at 26 °C of all.

Appendix D59

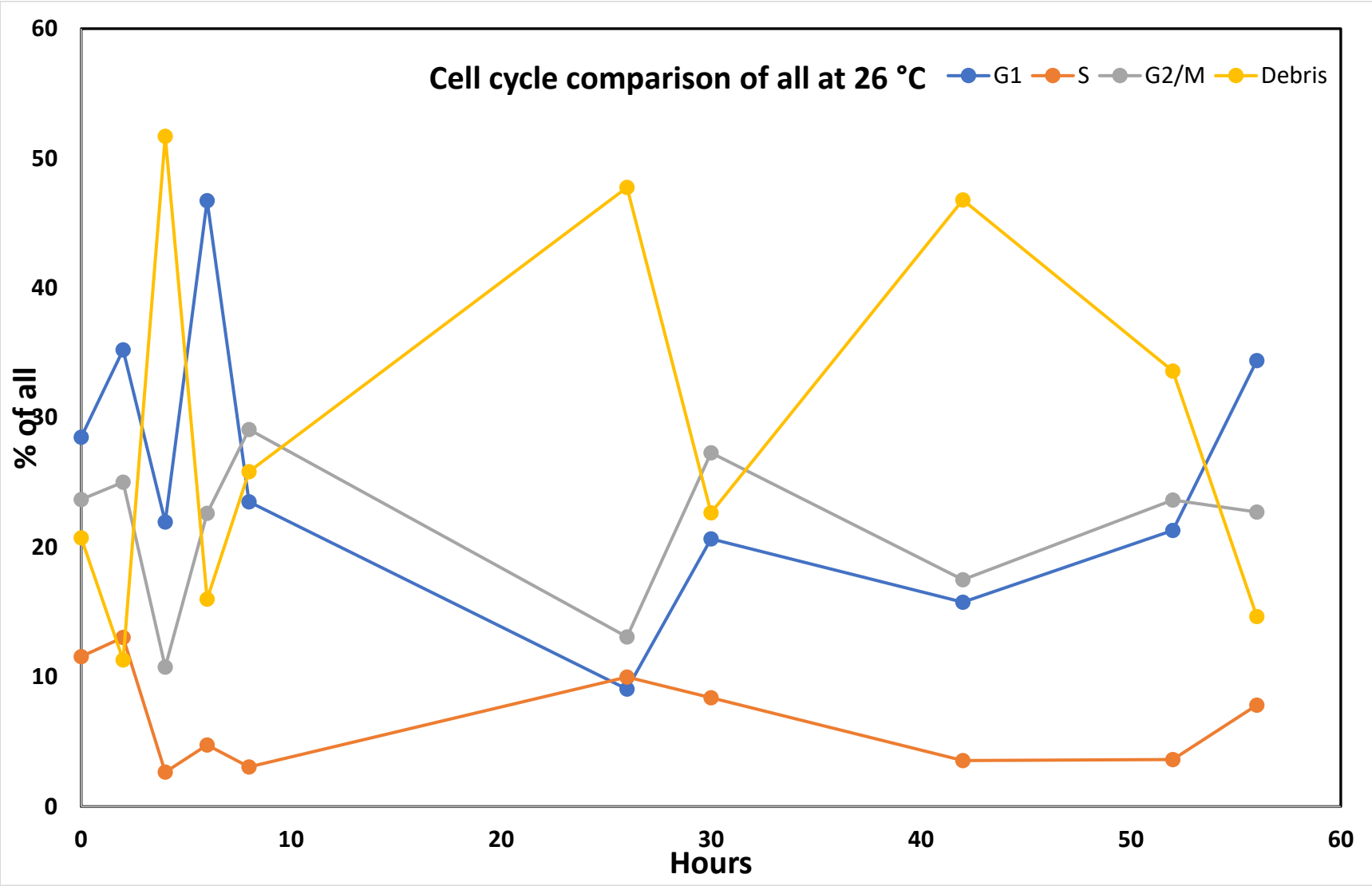


Figure 192. Chart of cell cycle at 26 °C of all.

Candidate number: 10014 og 10015

Optimisation of a Protocol for the Analysis of Cell Cycle in CHSE-214 Cells Using Flow Cytometry

NTNU

Kunnskap for en bedre verden