NovoCyte™ Flow Cytometer

Application Note No. 6

ACEA Biosciences, Inc.

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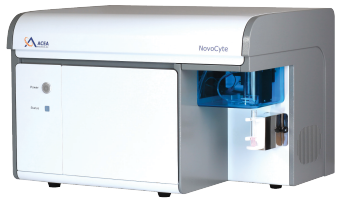
Proliferation and cell cycle analysis using the NovoCyte flow cytometer



The NovoCyte is a powerful and state of the art benchtop flow cytometer which can detect up to 13 colors and 15 total parameters. The system is optimally designed for quantitative and multi-parametric analysis of cellular events such as proliferation, viability, cell cycle and apoptosis. In this application note we will discussed how the NovoCyte System can be used for automated cell cycle analysis.

Eukaryotic cell division is an evolutionary conserved process where specific cellular components are duplicated in order to be divided equally amongst daughter cells. The precise timing and regulation of cell division is essential to many normal physiological processes and studying the molecular mechanisms regulating cell division is central to understanding both physiological and pathophysiological events, such as cancer. Once a cell becomes committed to undergoing cell division, it has to progress through distinct and elaborate steps of the cell cycle that are regulated by key signaling proteins which function as gate keepers. These steps are divided into three phases that can be easily detected using flow cytometry. Immediately following cell division, the cell enters the G1 phase where cellular growth occurs, including the replication of organelles followed by transitioning into the S phase of the cell cycle, in which DNA is duplicated, allowing progression into the G2/M phase. This is a phase of rapid cell growth and protein synthesis to prepare for mitosis, the final step where cellular components are divided equally and division occurs, producing two identical cells. This process repeats itself as cells continue to proliferate.

In order to analyze the specific phases of the cell cycle with the NovoCyte Flow Cytometer, Jurkat T cells were treated with different compounds known to induce growth arrest at G1 and G2/M. Subsequent to compound treatment, the cells were fixed with ice-cold 70% ethanol and labeled with propidium iodide, which binds stoichiometrically to DNA and allows for distinguishing varying amounts of DNA within cells. As shown in Figure 1, treatment with a compound known to arrest cells in G1 phase of the cell cycle, 5-Fluorouracil, culminated in a significant increase (17.85%) in the population of cells accumulating in the G1 phase, compared to the untreated control. Paclitaxel is an antimitotic compound and arrests the cells at the G2/M transition. Also shown in Figure 1, Paclitaxel treatment of Jurkat cells led to a 52.7% increase in the population of cells in the G2/M phase of the cell cycle as analyzed by propidium iodide staining. These results clearly demonstrate that the NovoCyte System can quantitatively detect cell populations existing in different phases of the cell cycle.

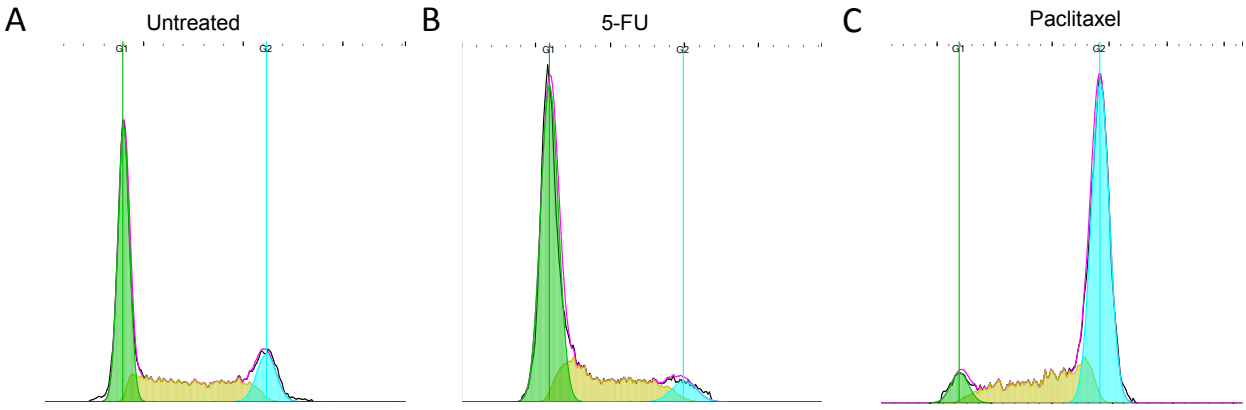


Figure 1. Cell cycle analysis in response to various treatments. Jurkat T cells were left A) untreated or treated with either B) 500uM 5-Fluorouracil (5-FU) or C) 10nM Paclitaxel for 16hr. Following treatment, the cells were fixed with ethanol and DNA content was measured using Propidium Iodide. Analysis was performed on the NovoCyte flow cytometer and automatic cell cycle analysis plots were applied.

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The end result of cell cycle progression is cell division culminating in an increase in cell number or proliferation. Specific reagents have been developed to allow measurement of cell proliferation in bulk or to study cells as they enter the different stages of the cell cycle. In order to study proliferation, we used the fluorescent dye CFSE. When cells labeled with CFSE divide, the dye is partitioned equally between daughter cells and the loss of CFSE fluorescence can be measured over time as the dye is continuously diluted. Jurkat T cells were labeled with CFSE and measurements of CFSE fluorescence were obtained every 24 hours. As shown in Figure 2A, the CFSE dye is serially diluted every time a measurement is taken by the NovoCyte, demonstrating that the cells are dividing and proliferating. The mean fluorescence intensity (MFI) was also plotted with cell concentration over time to show the inverse relationship between the extent of fluorescence and cell proliferation. (Fig. 2B)

The cell cycle analysis module included with the NovoExpress flow cytometry software facilitates quick and easy generation of cell cycle data using the Watson model. It is as simple as identifying your cells of interest, gating on single cells, and selecting the automatic cell cycle histogram plot icon. All relevant information is automatically calculated and displayed such as percent of cells in each stage of the cycle, CV's, and G2:G1 size ratio.

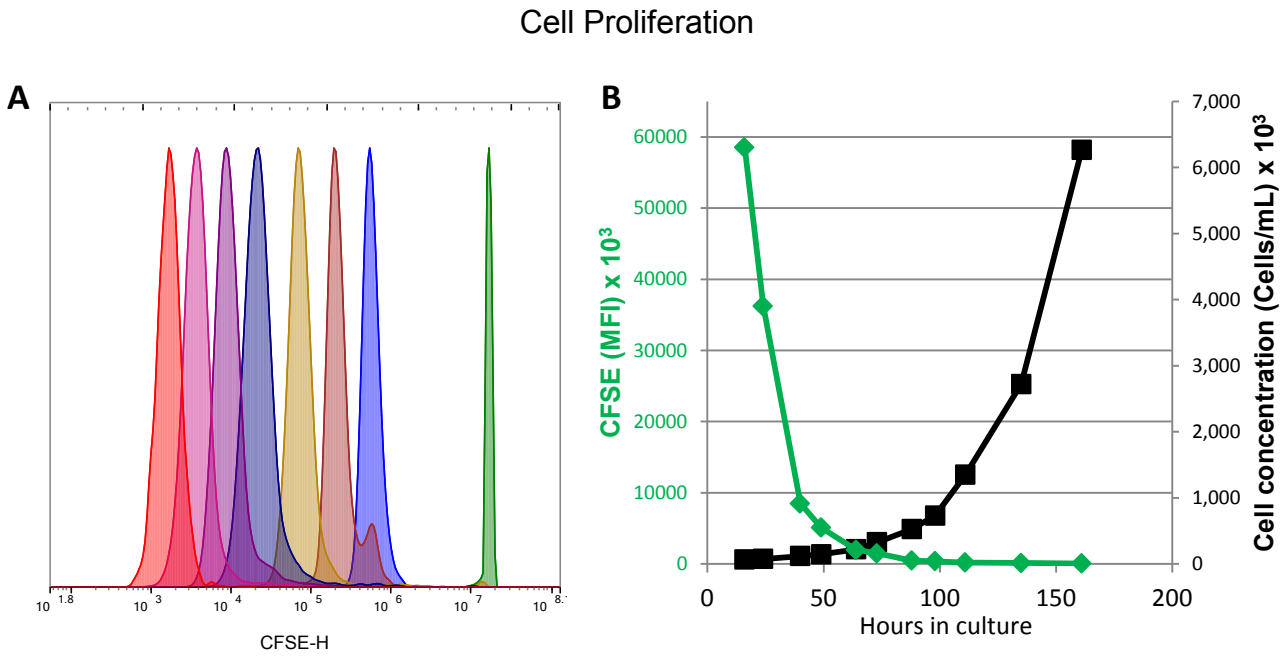

Figure 2. Measurement of proliferation in Jurkat T cells using CFSE. A) Jurkat T cells were labeled with CFSE and analyzed on the NovoCyte flow cytometer over time to measure cell division. Each peak is representative of an individual time point. B) Absolute cell counts are plotted alongside mean fluorescence intensity (MFI) of CFSE over time showing the dilution of signal as cells divide.

References

Darzynkiewicz, Z, Nucleic Acid Analysis. In: Robinson, JP, managing editor. Current Protocols in Cytometry. New York : J Wiley & Sons, Inc; 1997. Chapter 7.
Lyons, A.B. Analysing cell division in vivo and in vitro using flow cytometric measurement of CFSE dye dilution. J. Immunol. Methods 243, 147-154 (2000).

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