Application Note

Simplified Cell Cycle Phase Analysis with the Cytek® Muse® Cell Cycle Assay

Cell Cycle Regulation: Key to Cell Health and Proliferation

The cell cycle represents one of the most significant and fundamental processes in eukaryotic cells, resulting in cell growth and division into two daughter cells.

The regulation of cell cycle is critical to cell survival, as it governs the repair of genetic damage and the prevention of uncontrolled cell division. Defects in cell cycle regulation are a characteristic feature of tumor cells, and mutations in the genes involved in controlling the cell cycle are extremely common in cancer. Cell cycle analysis has become increasingly important in the understanding of the actions of anticancer compounds and studying mechanisms of cell division.

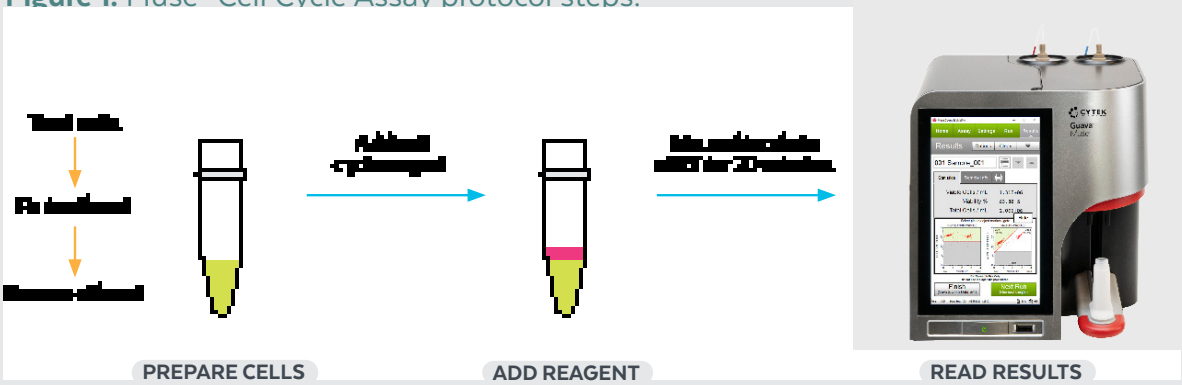
The Cytek® Muse® Cell Cycle Assay allows for the facile, rapid, and quantitative measurements of a percentage of the cells in the G0/G1, S, and G2/M phases of cell cycle on the Cytek® Muse® cell analyzer. The assay simplifies an analysis that has traditionally required complicated instrumentation and training, and enables users to easily obtain information on cell cycle distribution on their benchtops.

Principle of the Assay

The Muse Cell Cycle Assay uses the nuclear DNA stain propidium iodide (PI) to distinguish cells at different stages of the cell cycle, which differ in DNA content. Resting cells (G0/G1) contain two copies of each chromosome. As cells begin cycling, they synthesize chromosomal DNA (S phase). Fluorescence intensity from the DNA intercalating dye, PI, increases until all chromosomal DNA has doubled (G2/M phase).

At this state, the G2/M cells fluoresce with twice the intensity of the G0/G1 population. The G2/M cells eventually divide into two cells. The assay, thus, utilizes the differential staining of cells based on DNA content. Ethanol-fixed cells are treated with a premixed Muse Cell Cycle Reagent and acquired using the Muse Cell Cycle software module (**Figure 1**).

Figure 1. Muse® Cell Cycle Assay protocol steps.



Cytek Biosciences, Inc. 47215 Lakeview Blvd, Fremont, CA 94538 1-877-922-9835 sales@cytekbio.com

Muse Cell Cycle Assay Features and Benefits

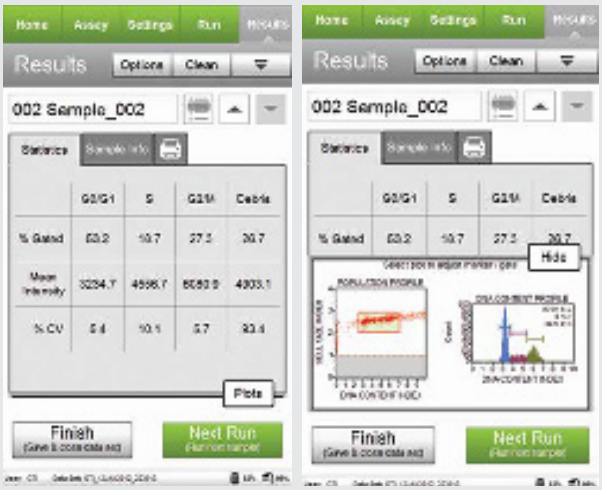
- Simplified protocol allows for quick determination of cells in all three phases of the cell cycle
- Premixed single reagent means no reagent preparation
- User guided touchscreen highly simplifies acquisition and analysis
- Minimal number of cells required facilitates running a larger number of samples or saving precious sample for other experiments
- Optimized reagent flexibility works with both adherent and suspension cells
- Flow cytometry principles enable accurate and precise results

Touchscreen Interface Greatly Simplifies Cell Cycle Data Acquisition and Analysis

The Muse Cell Cycle Software Module guides you through setup, acquisition, and analysis in a few simple steps.

- Intuitive touchscreen guides users to the answers
- Results include percentage of populations automatically displayed after acquisition, and a histogram with three markers to demarcate the G0/G1, S, and G2/M cell cycle phases
- Easy raw data and Excel® export features allow for archiving of results and additional analysis

Figure 2.



Results obtained for Jurkat cells stained with the Muse® Cell Cycle Kit, acquired on the Muse cell analyzer, and analyzed with the Muse Cell Cycle Software Module.

Versatile and Accurate

The Muse Cell Cycle Assay can be used for studying the impacts of cell cycle with a variety of treatment conditions and compounds (**Figure 3**). The assay works well with both suspension and adherent cell types (**Figures 3** and **4**). Results obtained from the simple, easy to use Muse cell analyzer are equivalent to those from traditional analysis methods, such as the Cytek® Guava® personal cell analyzer (PCA) flow cytometry system, as shown in **Table 1**.

Table 1

	G0/G1	S	G2/M
Muse® Cell Analyzer	50.31 ±1.54	30.3 ±0.48	19.39 ±1.61
Guava® PCA	47.79 ±1.00	33.79 ±1.20	18.42 ±0.25

Population percentages for the Muse cell analyzer compared to the Guava® PCA flow cytometry system. Percentages shown are averages of three individual samplings and their standard deviations.

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Figure 3.

Impact of cell-cycle-disrupting compounds on Jurkat cells analyzed using the Muse Cell Cycle Assay. Nocodazole, a microtubule disrupter, leads to cell cycle arrest in G2/M phase; etoposide, a known anticancer compound, also causes G2/M arrest.

Untreated Control
DNA Content Profile

Nocodazole
DNA Content Profile

Etoposide
DNA Content Profile

Figure 4.

Cell cycle profiles of MCF-7 (A) and PC-3 (B) cells obtained with the Muse Cell Cycle Assay.

MCF-7 Applicable to Multiple Types
DNA Content Profile

PC3 Applicable to Multiple Types
DNA Content Profile

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