



Muse™ Cell Cycle Kit User's Guide

Catalog No. MCH100106 (100 Tests)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

Application

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.¹ The regulation of the 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 understanding the action of anti-cancer compounds² or studying mechanisms of cell division.³

The Muse™ Cell Cycle Kit allows for the facile and rapid quantitative measurements of the percentage of cells in the G0/G1, S, and G2/M phases of cell cycle on the Muse™ Cell Analyzer. The assay allows you to easily obtain information on cell cycle distribution on your benchtop. The Muse™ Cell Cycle Kit quantitates the cell cycle phases in cell suspensions from a variety of cultured mammalian cell lines.

Data generated with the Muse™ Cell Cycle software module provides:

- percent of total cells for each phase of the cell cycle (G0/G1, S, and G2/M)
- mean fluorescence intensity
- %CV of fluorescence intensity

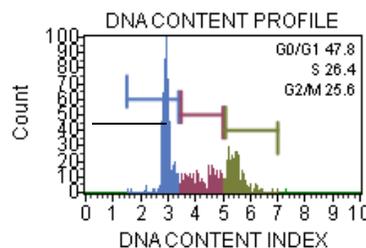
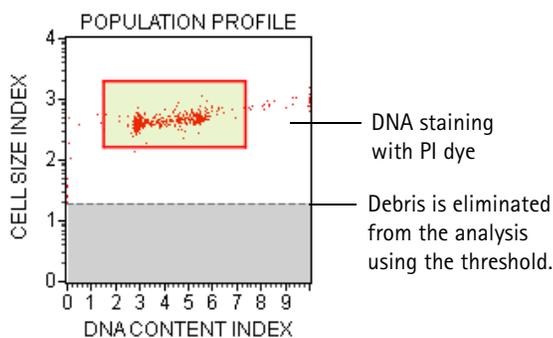
Sufficient reagent is provided for the preparation and analysis of 100 tests.

Test Principle

The Muse™ Cell Cycle Assay uses a premixed reagent™ which includes the nuclear DNA intercalating stain propidium iodide (PI) and RNase A in a proprietary formulation. PI discriminates cells at different stages of the cell cycle, based on differential DNA content in the presence of RNase to increase the specificity of DNA staining.⁴⁻⁷ Resting cells (G0/G1) contain two copies of each chromosome. As cells begin cycling, they synthesize chromosomal DNA (S phase). Fluorescence intensity from PI increases until all chromosomal DNA has doubled (G2/M phase). At this stage, 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 utilizes PI-based staining of DNA content to discriminate and measure the percentage of cells in each cell cycle phase (G0/G1, S, and G2/M).

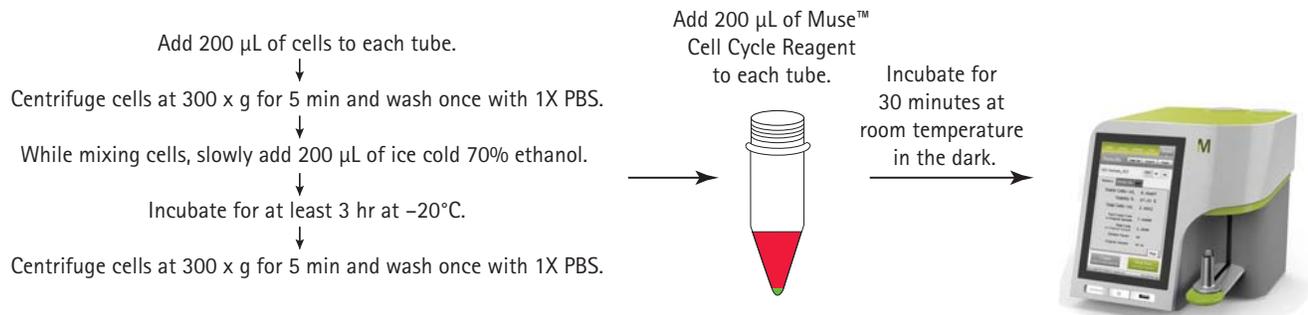
The Muse Cell Cycle Software Module performs calculations automatically. Data is displayed in two plots:

- DNA content index and cell size index dot plot
- DNA content index histogram with markers available to analyze the cell populations in each phase of the cycle



Percentages of cells in G0/G1, S, and G2/M phases of the cell cycle

Summary of Protocol



Kit Components

Muse™ Cell Cycle Reagent (Part No. 4700-1495, 100 tests/bottle)

Materials Required but Not Supplied

- Muse™ Cell Analyzer
- Cell suspension
- 100% ethanol
- Dilution buffer: Phosphate buffered saline (PBS), or equivalent balanced salt solution, pH 7.2 to 7.4
- Micropipettors
- Disposable micropipettor tips
- 12 x 75-mm polystyrene tubes (VWR Catalog No. 60818-270)
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog No. 16466-030, or equivalent)
- Vortex mixer
- Disposable gloves
- 20% bleach solution
- Deionized water
- Centrifuge
- Muse™ Count & Viability Kit (Catalog. No. MCH100102 (100T) or Catalog. No. MCH600103 (600T)), optional
- Muse™ Cell Dispersal Reagent (Catalog No. MCH100107), optional
- Guava® ICF Instrument Cleaning Fluid (Catalog No. 4200-0140), optional
- Muse™ System Check Kit (Catalog No. MCH100101)

Precautions

- The Muse™ Cell Cycle Kit is intended for research use only.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
- The Muse™ Cell Cycle Reagent contains dyes that may be carcinogenic and/or mutagenic. Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic and mutagenic reagents. Refer to the MSDS for specific information on hazardous materials.

- The Muse™ Cell Cycle Reagent contains sodium azide, which is toxic if ingested. Reagents containing sodium azide should be considered a poison. If products containing sodium azide are swallowed, seek medical advice immediately and show product container or label. (Refer to NIOSH, National Institute for Occupational Safety and Health; CAS#: 2628-22-8; and also to GHS, The Globally Harmonized System of Classification and Labeling of Chemicals.) Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas. Any reagents containing sodium azide should be evaluated for proper disposal. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent build-up in plumbing. Check with regulatory agencies to determine at what concentration sodium azide may cause a product to be regulated as hazardous.
- Avoid microbial contamination of the solution, which may cause erroneous results.
- All biological specimens and materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
- Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
- The fluorescent dye in this reagent is light sensitive. Store at 2 to 8°C in the dark and shield from excessive exposure to light.
- The instructions provided have been designed to optimize the kit's performance. Deviation from the kit's instructions may result in suboptimal performance and may produce inaccurate data.
- During storage and shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For maximum recovery of the product, centrifuge the vial briefly prior to removing the cap.
- Do not use the reagent beyond the expiration date.
- Material Safety Data Sheets (MSDS) for kit reagents are available from our website (www.millipore.com/muse), by contacting Millipore Technical Support, or from the Millipore technical library at www.millipore.com/techlibrary.

Storage

- Store the Muse™ Cell Cycle Kit refrigerated at 2 to 8°C. Do not freeze. Refer to the expiration date on the package label. Do not use the reagent after the expiration date.
- The Muse™ Cell Cycle Reagent contains light-sensitive dyes. Shield from excessive exposure to light.

Before You Begin

It is highly recommended that you run the cell samples shortly after completing the sample preparation. While some cell types have been shown to yield stable results for up to 24 hours after staining with the Muse™ Cell Cycle Reagent, the stability of individual cell types may vary.

Time considerations: Prior to staining, cell fixation requires at least 3 hours, and preparing cells for testing may require periodic maintenance and cultivation several days in advance. The process of staining cells with the Muse™ Cell Cycle Reagent and the recommended protocol takes 40 to 45 minutes. Acquiring data on your Muse™ Cell Analyzer takes less than 3 minutes per sample, depending on the cell concentration and desired number of events to acquire.

Always run a System Check prior to performing the assay. For details refer to the *Muse™ Cell Analyzer User's Guide*.

Cell Sample Fixation in Tubes

Preparing Fixative Solution

Prepare fresh 70% ethanol.

NOTE: Due to the stability of 70% ethanol, we recommend making fresh, cold 70% ethanol using high-grade absolute ethanol on the cell fixation day.

1. Mix the cold absolute ethanol (200 proof) with cold DI water. See the following table for amounts. Store 70% ethanol at -20°C until use. Keep 70% ethanol on ice at all times during usage.

NOTE: It is essential to keep the fixative very cold for optimal fixation and high quality cell cycle resolution.

Ethanol Preparation Instructions

	For 1 mL of 70% EtOH (1 sample)	For 2 mL of 70% EtOH (2 samples)	For 5 mL of 70% EtOH (5 sample)	For 10 mL of 70% EtOH (10 samples)
Volume of cold 100% EtOH	0.7 mL	1.4 mL	3.5 mL	7 mL
Volume of cold DI water	0.3 mL	0.6 mL	1.5 mL	3 mL

2. Proceed to "Fixing Samples" in the following section.

Fixing Samples

It is important to have a single cell suspension prior to ethanol fixation. Otherwise, the ethanol fixation process will result in a high percentage of aggregated cells and/or debris, affecting the accuracy of your results.

1. Transfer the cell sample to a 12 x 75-mm polystyrene tube or 15-mL or 50-mL conical tube (depending on the total cell number) if the cells are not already in a tube. The minimum recommended number of cells for fixation in a tube is 1×10^6 cells.
2. Centrifuge the tube at 300 x g for 5 minutes.
3. Remove and discard the supernatant without disturbing the cell pellet.
After centrifugation, the cell pellet forms either a visible pellet or a white film on the bottom of the tube.
4. Add appropriate volume of PBS to each tube (ie, 1 mL of PBS per 1×10^6 cells). Mix the cells well by pipetting several times or gently vortexing.
5. Centrifuge the cells at 300 x g for 5 minutes.
6. Remove and discard the supernatant without disturbing the cell pellet. Leave approximately 50 μL of PBS per 1×10^6 cells.
7. Resuspend the cell pellet in the residual PBS by repeated pipetting several times or gently vortexing.
8. Add the resuspended cells drop-wise into the tube containing 1mL of ice cold 70% ethanol while vortexing at medium speed.
9. Cap and freeze the tube at -20°C for at least 3 hours prior to staining. Fixed cells are stable for 2 to 3 months at -20°C .
10. Proceed to the "Staining Protocol" protocol in the following section.

Staining Protocol

1. Obtain a uniform ethanol-fixed cell suspension for staining. For more information on cell preparation and fixation see "Cell Sample Fixation in Tubes" above and Cell Preparation section in Appendix A.

NOTE: If cells appear to be in clumps, see "Troubleshooting" on page 12 to determine how to proceed.

2. Add 200 μL of ethanol-fixed cells to a 12 x 75-mm polystyrene test tube. The cell concentration should be between 5×10^5 to 1×10^6 cells/mL.

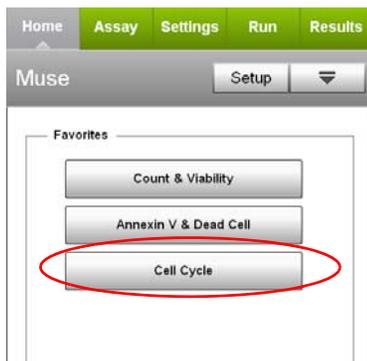
NOTE: We recommend using 12 x 75-mm polystyrene tubes to minimize cell loss.

3. Centrifuge ethanol-fixed cells at 300 x g for 5 minutes at room temperature.
4. Remove and discard the supernatant.
5. Resuspend the cell pellet in 0.25 mL PBS per 5×10^5 cells.
6. Centrifuge cells at 300 x g for 5 minutes at room temperature.
7. Remove and discard the supernatant.
8. Resuspend the cell pellet in 200 μL of Muse™ Cell Cycle Reagent.
9. Incubate for 30 minutes at room temperature, protected from light.
10. Transfer cell suspension sample to a 1.5-mL microcentrifuge tube prior to analysis on Muse™ Cell Analyzer.

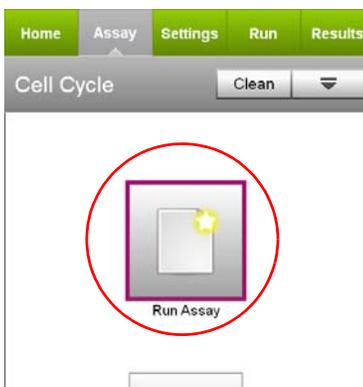
Setup and Acquisition on the Muse™ Cell Analyzer

Run a System Check prior to performing the assay. For information on Muse™ System Check, refer to the *Muse™ Cell Analyzer User's Guide*.

1. Select **Cell Cycle** from the main menu.

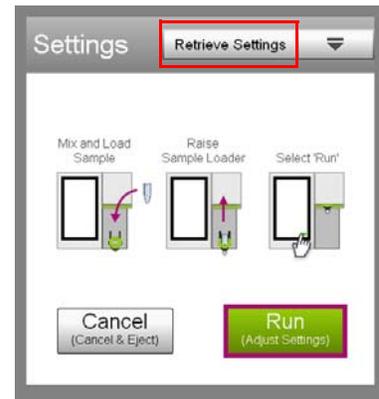


2. Select **Run Assay**.



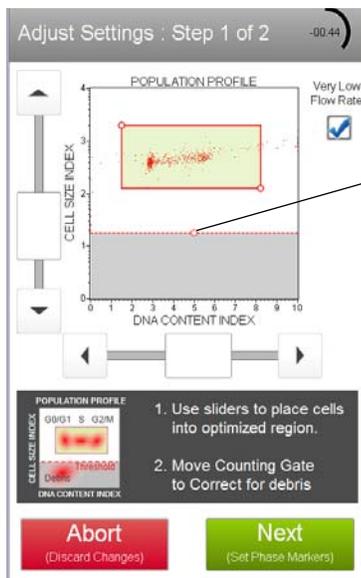
3. Adjust the instrument settings.

- Load a stained sample for adjusting the settings and select **Run**.
- Or, to retrieve previously saved instrument settings, select **Retrieve Settings**. For more information on retrieving settings, see the *Muse™ Cell Analyzer User's Guide*.

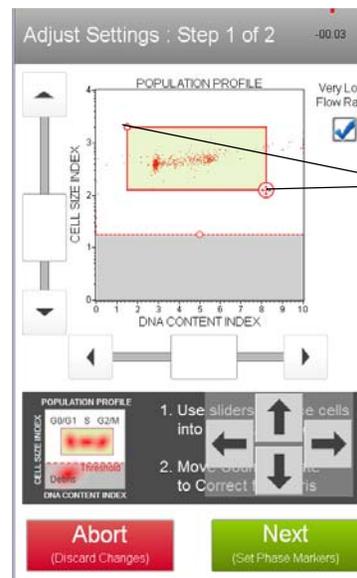


4. Fine tune the settings for the DNA CONTENT INDEX vs CELL SIZE INDEX plot, if necessary.

- Very Low Flow Rate is selected by default. If you uncheck the box, a Low Flow Rate will apply, resulting in an increase of the %CV of G0/G1, S, and G2/M cell cycle peaks.
- Adjust the CELL SIZE INDEX slider to the left of the plot and the DNA CONTENT INDEX slider below the plot to move the cellular populations into the green gated region.
- Drag the threshold to exclude debris. Drag the threshold marker to make large changes. Use the arrow buttons below the plot to make small changes. The arrow buttons appear after you touch the threshold.
- Touch the upper-left and/or lower-right corners of the gate and drag to adjust the gate to include the cells of interest.



Touch circle to drag the threshold up or down.



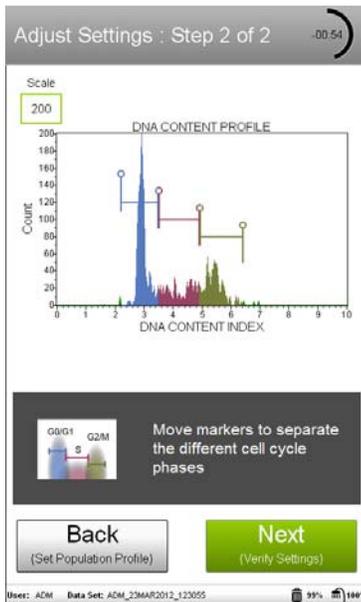
Touch upper-left or lower-right corner to adjust the gate.

NOTE: If the acquisition times out (after 2 minutes), you can select **Back** to restart the adjust settings step or **Next** to accept the settings and continue to the next step. If acquisition times out, remove the tube and mix well before reloading and continuing.

5. Select **Next** when you've completed the adjustments.

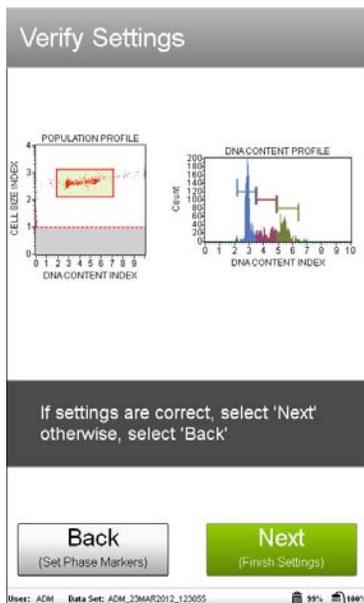
6. Fine tune the settings for the DNA CONTENT PROFILE histogram, if necessary.

Adjust all four markers to distinguish the G0/G1 (blue), S (purple), and G2/M (green) cells. Touch and drag the individual markers, or touch a marker and use the arrow buttons to adjust the position. Be sure to exclude all debris.

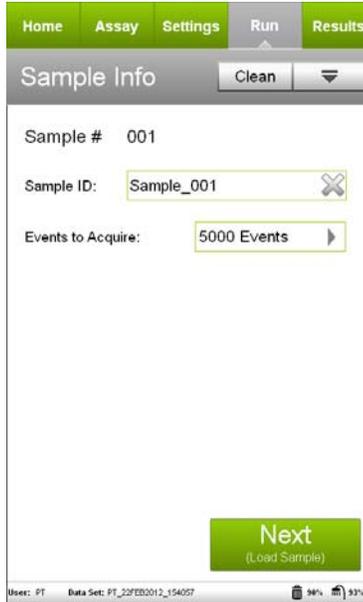


7. Select **Next** when the marker adjustments are complete.

8. Verify the settings. If the settings are correct, select **Next**. Otherwise, select **Back** and repeat steps 4 through 7, as necessary.



9. Enter the sample ID by touching the field, then using the keypad to input the ID. Touch **Done** when you've finished entering the ID. If necessary, change the Events to Acquire by touching the field, then selecting the value from the pop-up menu. Select **Next**.

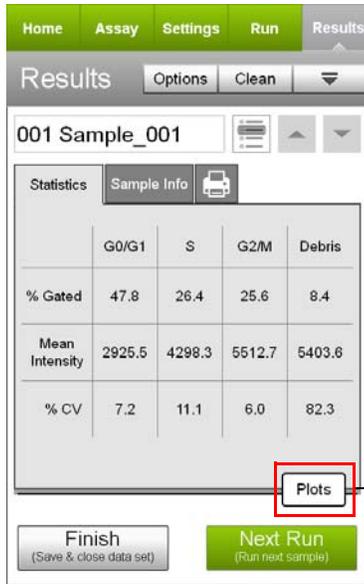


10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.

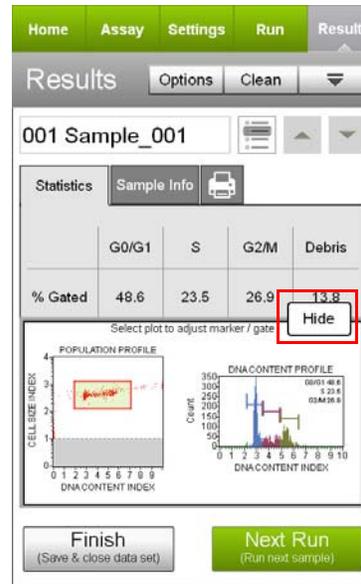


11. When acquisition is complete, the results are displayed. If desired, select **Plots** to display a dot plot and histogram for the sample. See "Results" on page 10 for more information.

You can view or change the sample ID, as well as add annotations for the current sample by selecting the Sample Info tab. To print the results for the current sample select the printer tab.



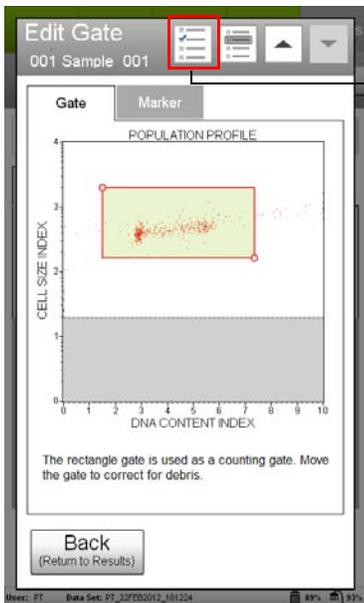
Select to display plots.



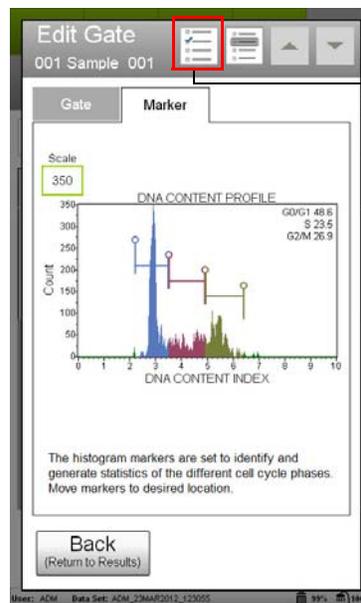
Select to hide plots.

12. (Optional) If changes are needed to the gate or markers, touch a plot to enlarge it, then adjust the cell size gate or markers, as described in steps 4 and 6, respectively. You cannot adjust the cell size threshold after the sample has been acquired.

If you adjust the gate or marker on subsequent samples and wish to apply the changes to other samples that you already acquired, select the Apply Changes button () in the title bar. Select the samples you want to apply the changes to or choose **Select All**, then select **Apply**. The sample you originally made changes to must be selected.



Apply changes



Select to apply changes to other samples.

13. If no adjustments are needed, select **Next Run** and repeat steps 9 through 12 for the remaining samples.

NOTE: During the run, a message may appear prompting you to load a tube of DI water for a Quick Clean. Load the water then select **Clean** to perform the Quick Clean. Select **Next** to continue with the run. The frequency of Quick Cleans was set by your system administrator. Your administrator may also have chosen to allow you to skip the Quick Clean when the prompt appears. You can choose to perform additional Quick Cleans at any time during a run by selecting **Clean** in the title bar, then **Quick Clean** from the menu.



14. When you have acquired the last sample, select **Finish**.

15. (Optional) Select **Options** in the title bar to rename the data set, export the data set, save the current instrument settings, or view the event log. Refer to the *Muse™ Cell Analyzer User's Guide* for more information.

Results

The software performs calculations and displays the data in two plots:

- a dot plot displaying DNA Content Index vs Cell Size Index for setting the gate
- a histogram displaying DNA Content Index vs Count for assessing the percentage of cells in each phase

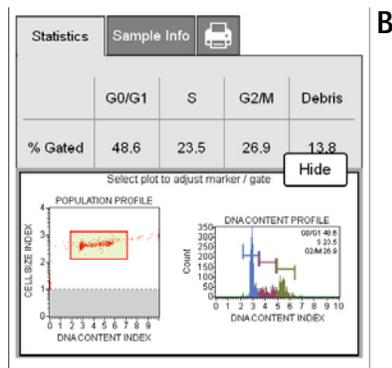
Results from each run are stored in a data file, as well as its corresponding spreadsheet (CSV) file. The data file and spreadsheet file contain the following statistics:

- sample number
- sample ID
- percentage of cells in G0/G1, S, and G2/M phases, and debris
- mean fluorescence intensity of G0/G1, S, and G2/M phases, and debris
- %CV of G0/G1, S, and G2/M phases, and debris

A

Statistics		Sample Info			
	G0/G1	S	G2/M	Debris	
% Gated	47.8	26.4	25.6	8.4	
Mean Intensity	2925.5	4298.3	5512.7	5403.6	
% CV	7.2	11.1	6.0	82.3	

Plots



Figures A and B. Jurkat cells were stained with the Muse™ Cell Cycle Kit and acquired on the Muse™ Cell Analyzer. Figure A shows summary data, while Figure B shows results displayed with the optional dot plot and histogram. The statistics show the percentage, mean fluorescence intensity, and %CV of each cell cycle phase (G0/G1, S, and G2/M), as well as debris. The first plot in Figure B displays DNA Content Index vs Cell Size with a gate around the cellular population and debris appearing to the left of the gate. The second plot in Figure B shows the distribution of gated cells within each phase, with debris or sub G0/G1 cells to the left of the first marker and aggregates or G4 cells to the right of the last marker.

Technical Tips

1. Setting the Cell Size Index Threshold too low may affect your results because debris will be included. As a result, noise may appear in the DNA Content Index histogram. Try increasing the threshold to reduce the noise and the unwanted debris.
2. Avoid excessive exposure of the stained samples to the light.
3. If the concentration of the stained cell sample for data acquisition is low ($<7 \times 10^4$ cells/mL), the Muse™ Cell Analyzer will not be able to acquire 5000 events in the time allotted for sample collection. Centrifuge the sample at $300 \times g$ for 7 to 10 minutes and remove a sufficient volume of supernatant to increase cell concentration to $>7 \times 10^4$ cells/mL.
4. If the cell concentration is greater than 1200 cells/ μL , a warning message appears during acquisition. Dilute the cell sample with Muse™ Cell Cycle Reagent.
5. Cell fixation, washing, and cell cycle staining should be done in 12 x 75-mm polystyrene tubes. We do not recommend using 1.5-mL microcentrifuge tubes for cell washing and staining.
6. For consistent and accurate results, mix each cell sample thoroughly on a mixer before acquiring. However, avoid vigorous mixing, which can cause the solution to splash out of the tube, and cellular breakdown resulting in volume loss and erroneous results.
7. Multiple acquisitions of a cell sample minimize sampling error. Statistically, multiple acquisitions yield more accurate results.
8. The default number of events to acquire is 5000. You may input a different number; however, your statistical error will increase as you decrease the number of events for acquisition.
9. Periodically run Quick Clean using a tube of DI water (after every 12 sample acquisitions) to prevent a buildup from cellular debris in the system. If your samples contain significant amounts of cellular debris, run the Quick Clean cycle more often to prevent clogs or blockage.
10. If you are acquiring data from a sample but the progress bar is not moving, there is probably either insufficient volume to continue to acquire the sample or a blockage of the flow system. First check to ensure that there is at least 150 μL of sample in the tube. If not, add additional Cell Cycle Reagent to bring the volume up to 150 μL or proceed to the next sample. If the sample volume is greater than 150 μL , then the lack of events is probably due to a clog. A clog or blockage can be caused by cell aggregates, cell debris, bleach crystals, or other particulates. Perform a Backflush to flush out the clog into a tube containing 20% bleach. Then run a Quick Clean with DI water to remove bleach residue. If this procedure does not alleviate the problem, refer to the *Muse™ Cell Analyzer User's Guide* for additional troubleshooting tips, or contact Millipore Technical Support for help.
11. Cell Cycle works best with samples in a homogeneous, single-cell suspension. Cell aggregates may clog the flow cell or be excluded from the gate because they are thought to be G4 cells, affecting the accuracy of your results. For more troubleshooting tips, refer to the *Muse™ Cell Analyzer User's Guide*.

For more information, contact the Millipore office nearest you. In the US, call 1-800-MILLIPORE (1-800-645-5476). Outside the US, visit our website at www.millipore.com/offices for up-to-date worldwide contact information. You can also view the tech service page on our web site at www.millipore.com/techservice.

Troubleshooting

Potential Problem	Experimental Suggestions
Acquisition taking longer than expected or progress bar stops during acquisition	Ensure that the System Check procedure was run and passed. If the progress bar stops during acquisition, the fluid system may be clogged. Run a Quick Clean procedure to clean the capillary. It can be performed during or after an assay.
Instrument clogging	If the instrument is clogged, run a Quick Clean procedure to clean the capillary. It can be performed at anytime during an assay between samples.
Visible cell aggregates	Filter cell sample through a 40- μm nylon mesh immediately prior to running on the Muse™ Cell Analyzer. Doublet cells with 2n DNA content (G1 phase) each have the same DNA content as a single cell with 4n DNA content (G2/M phase).
Low Cell Concentration warning during acquisition	Ensure that cells are counted properly prior to beginning the experiment. The assay instructions are optimized to give you a range of cells between 300–700 cells/ μL in the final sample volume so accurate population count results are obtained. A substantial decrease in cell numbers can lead to difficulty in adjusting settings.
High Cell Concentration warning during acquisition	If the concentration of the stained cell sample is high (>1200 cells/ μL), dilute the sample further with Muse™ Cell Cycle Reagent to adjust the cell concentration between 300 and 700 cells/ μL .
High %CVs (wide peaks) or false peak	Although the assay procedure has been optimized to function for multiple cells types, every cell line behaves differently. The wide peaks or false peak may indicate that: <ul style="list-style-type: none"> • The instrument flow rate is set to Low instead of Very Low. Ensure the Very Low Flow Rate box is selected on the adjust settings screen. • The sample is poorly fixed and stained as a result of cell aggregates. Ensure your sample is a single-cell suspension before fixing and staining. • Cell concentration is too high. Decrease the number of cells by diluting the sample to 300–700 cells/μL. The Muse™ Cell Analyzer gives the most accurate data when the flow rate is between 300 and 700 cells/μL.
Low level of staining	<ul style="list-style-type: none"> • Although the assay procedure has been optimized to function utilizing multiple cell types, every cell line behaves differently. A low signal may indicate that the cells need to be stained at a higher volume. • Verify that the System Check procedure was performed and the results passed.
Variability in day-to-day experiments	<ul style="list-style-type: none"> • If the results are inconsistent, check that the samples were well mixed prior to acquisition. Cells may quickly settle in your samples and your results will be inaccurate unless the cells are mixed just prior to acquisition. • Monitor experimental cell cultures to ensure that cell viability and cell numbers being analyzed are consistent. Any drop in cell numbers or viability can influence experimental results. • If there appears to be day-to-day variation of the staining pattern, ensure the Muse™ Cell Analyzer is working properly. Check the Muse™ System Check log to ensure day-to-day instrument variation is low.

Limitations

The results of the assay are dependent upon proper handling of samples, reagents, and instruments.

Appendix A: Cell Sample Preparation

Preparing Non-Adherent and Adherent Cells

The following protocols describe how to harvest non-adherent or adherent cells cultured in 96-well plates, as well as non-adherent or adherent cells cultured in flasks or other tissue culture vessels. Each of the culturing conditions requires different protocols to harvest the cells.

Preparing non-adherent cells

1. Set up initial culture conditions, such that after culture and treatment, cells are at a concentration of 1×10^5 to 1×10^7 cells/mL in serum- or albumin containing medium.

NOTE: Include an untreated sample to use as a negative control.

2. Proceed to "Cell Sample Fixation in Tubes" on page 4.

Preparing adherent cells

For harvesting adherent cells, use your method of removal. Reagents such as EDTA or trypsin can be used to dissociate the cells from the flask and should create single-cell suspensions. If using mechanical means to dislodge the cells, additional reagents such as Muse™ Cell Dispersal Reagent (Catalog No. MCH100107) may be used to dissociate clumps.

1. Using your preferred method for dissociation, detach the cells from their culture vessel.
2. Add fresh serum- or albumin-containing medium to each well/tube so the final concentration is between 1×10^5 to 1×10^7 cells/mL.
3. Proceed to "Cell Sample Fixation in Tubes" on page 4.

References

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7. Ho J, Gillis K, Fishwild D. A simple and robust system for determining cells cycle distribution: the Guava cell cycle assay. Guava Technologies Application Note, 2004.

Related Kits

- Muse™ System Check Kit – MCH100101
- Muse™ Count & Viability Kit (100T) – MCH100102
- Muse™ Count & Viability Kit (600T) – MCH600103
- Muse™ Count & Viability Kit (200X) – MCH100104
- Muse™ Annexin V & Dead Cell Kit – MCH100105
- Muse™ Cell Dispersal Reagent – MCH100107

Warranty

EMD Millipore Corporation ("Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for either: a) a period of one year from the date of shipment of the products; or b) a term less than one year, as such term is expressly stated on the product specification or product label. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications, and descriptions of Millipore products appearing in Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized, and if given, should not be relied upon.

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