Muse™ Count & Viability Kit User's Guide

Catalog No. MCH100102 (100 Tests)
Catalog No. MCH600103 (600 Tests)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Application

The Muse™ Count & Viability Reagent allows the quantitative analysis of cell count and viability on the Muse™ Cell Analyzer. It is a rapid and reliable alternative to trypan blue exclusion. The Muse™ Count & Viability Reagent provides absolute cell count and viability data on cell suspensions from a variety of cultured mammalian cell lines. Both viable and non-viable cells are differentially stained based on their permeability to the DNA-binding dyes in the reagent. Data generated with the Muse™ Count & Viability Software Module provides:

- viable cell count (cells/mL)
- total cell count (cells/mL)
- % viability of sample

The Muse™ Count & Viability Reagent is for use with the Muse™ Cell Analyzer. The Muse™ System makes sophisticated fluorescent-based analysis fast, easy, convenient, and affordable. Sample preparation is minimal, and after loading samples onto the Muse™ Cell Analyzer, intuitive software provides detailed or summary analysis of your cell sample in a few short steps.

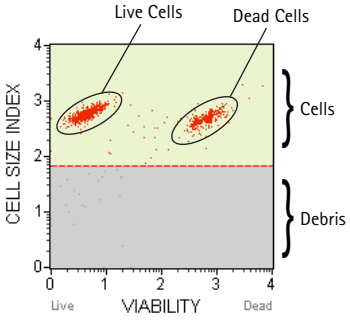
Sufficient reagent is provided for the preparation and analysis of 100 (MCH100102) or 600 (MCH600103) tests.

Test Principle

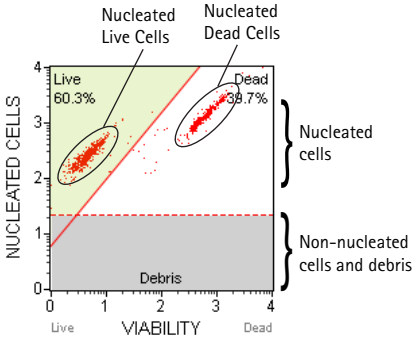
The Muse™ Count & Viability Reagent differentially stains viable and non-viable cells based on their permeability to the two DNA binding dyes present in the reagent. The Muse™ Count & Viability Software Module then performs calculations automatically and displays data in two dot plots.

- A DNA-binding dye in the reagent stains cells that have lost their membrane integrity, allowing the dye to stain the nucleus of dead and dying cells. This parameter is displayed as VIABILITY and is used to discriminate viable (live cells that do not stain) from non-viable (dead or dying cells that stain).
- A membrane-permeant DNA staining dye that stains all cells with a nucleus. This parameter is displayed as NUCLEATED CELLS and is used to discriminate cells with a nucleus from debris and non-nucleated cells. The Muse™ System counts the stained nucleated events, then uses the cellular size properties to distinguish free nuclei and cellular debris from cells to determine an accurate total cell count.

Dead Cell (Viability) Stain



Nucleated Stain



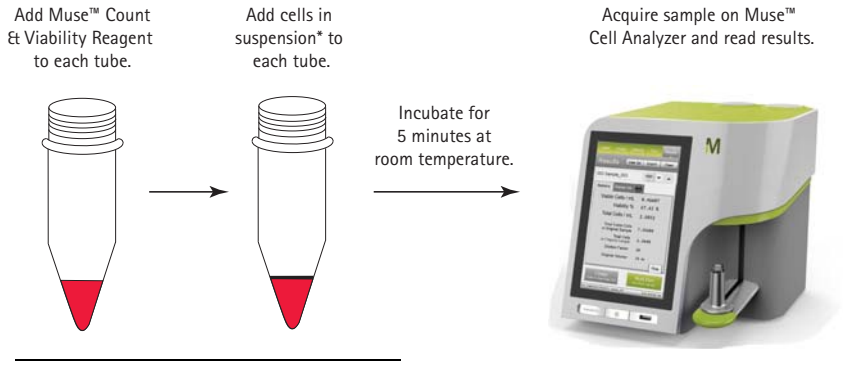
	Debris and Non nucleated cells	Viable Cells	Dying Cells	Dead Cells
Cell Size Index	Low	High	Mid to High	High
Nucleated Cell Stain	Neg	High	High	High
Viability Stain	Neg	Neg	Med	High

Nucleated cell stain differentiates cells from debris.

Dead Cell (Viability) stain differentiates viable (negative) from dead and dying cells (positive).

Each plot has moveable markers. The first plot has a gate marker, allowing you to eliminate debris based on size. The second plot also has a threshold marker, allowing you to eliminate cells that do not have a nucleus. This plot also has an angled marker (viability discriminator), allowing you to separate live cells from dead cells.

Summary of Protocol



* Adherent cells have been validated for this assay. For information on preparing adherent cells, see Appendix A.

Kit Components

Muse™ Count & Viability Reagent

- Catalog No. MCH100102, 100 tests
- Catalog No. MCH600103, 600 tests

Materials Required but Not Supplied

- Muse™ Cell Analyzer
- Cell suspension
- Dilution buffer: complete growth media or phosphate buffered saline (PBS), or equivalent balanced salt solution, pH 7.2 to 7.4.
- Micropipettors
- Disposable micropipettor tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog No. 16466-030, or equivalent)
- Vortex mixer
- Disposable gloves
- 20% bleach solution
- Deionized water
- 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), optional

Precautions

- The Muse™ Count & Viability Reagent is intended for research use only.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
- The Muse™ Count & Viability 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™ Count & Viability 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 result.
- 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.
- The fluorescent dyes in this reagent are light sensitive. Store in the dark and shield from excessive exposure to light.
- 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™ Count & Viability Reagent 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™ Count & Viability Reagent contains light-sensitive dyes. Shield from excessive exposure to light.

Before You Begin

This protocol was developed to allow direct count and viability determinations of multiple cell types. The kit provides reliable staining and results with cell concentrations in the range of 1×10^5 to 1×10^7 cells/mL.

It is highly recommended that cell samples be run shortly after the sample preparation has been completed. While some cell types have been shown to yield stable results for up to 4 hours after staining with the Muse™ Count & Viability Reagent, the stability of individual cell types may vary.

Time considerations: The cell staining procedure with the Muse™ Count & Viability Reagent takes 5 minutes. Acquiring data on your Muse™ Cell Analyzer takes less than 2 minutes per sample. However, preparing cells for testing may require periodic maintenance and cultivation several days in advance.

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

Staining Protocol

1. Prepare a uniform cell suspension for counting. Be sure adherent cells are completely removed from flasks and are well mixed. For more information on cell preparation see "Appendix A: Cell Sample Preparation" on page 12.

   NOTE: Accurate cell counting requires the even distribution of the cells within suspensions. Gently but thoroughly mix all suspensions during all dilution and staining steps and before loading samples onto the system for analysis. Do not vortex samples vigorously as sample can splash out, resulting in erroneous cell counts.

2. Prepare stained cell samples by mixing cells with Muse™ Count & Viability Reagent in a sample tube. Accurate cell counting occurs at a concentration range of 1 x 10^4 cells/mL to 5 x 10^5 cells/mL in the stained sample.

   - If you know the approximate concentration of the original cell suspension, refer to the following table as a dilution guide.

Cell Suspension Dilution Table (recommended volumes)

Concentration of Original Cell Suspension	Dilution Factor	Cell Suspension Volume	Count & Viability Reagent Volume	Concentration of Diluted Cells
1 x 10^5 to 1 x 10^6 cells/mL	10	50 µL	450 µL	<1 x 10^5 cells/mL
1 x 10^6 to 1 x 10^7 cells/mL	20	20 µL	380 µL	<5 x 10^5 cells/mL
1 x 10^7 to 2 x 10^7 cells/mL	40*	20 µL	780 µL	<5 x 10^5 cells/mL
* Further dilution may be necessary for highly concentrated cell suspensions.				

   - If you do not know the approximate concentration of your original cell suspension, prepare a stained cell sample by mixing with Muse™ Count & Viability Reagent at a 20-fold dilution (for example, 20 µL of cell suspension into 380 µL of Count & Viability Reagent).
   - If the original concentrations are >2 x 10^7 cells/mL, then results are out of the measurement range. Samples can be prepared with an 80-fold dilution of the original cell suspension and reacquired. Or, the original cell sample can be diluted with PBS or media before repeating the assay with the guidelines above. In this case, be sure to account for both dilutions by multiplying the dilution factors. For example, if you dilute your original sample 1:10 with PBS, then dilute the sample 1:10 with Count & Viability reagent during sample preparation, the final dilution factor is 100.
   - If the final concentration of the stained cell sample for data acquisition is too concentrated (>5 x 10^5 cells/mL), the cell count may not be accurate.

   Suggestion

   For highly concentrated cell suspensions (>1 x 10^7 cells/mL), we recommend that you start with a 40-fold dilution of your original cell suspension. You may need to further dilute the sample to attain a stained cell sample in the concentration range for accurate cell counting. For example, for cell suspensions of concentrations >2 x 10^7, you should prepare an 80-fold dilution of your original cell suspension. If you do not know the approximate concentration of your original cell suspension, prepare a stained cell sample by mixing with Count & Viability Reagent at a 20-fold dilution (for example, 20 µL of cell suspension into 380 µL of Count & Viability Reagent). If your cell sample shows a Total Cells per mL value of 1 x 10^7 or higher, we recommend that you prepare additional dilutions (for example, 40- and 80-fold dilutions) for the assay to verify the cell count.

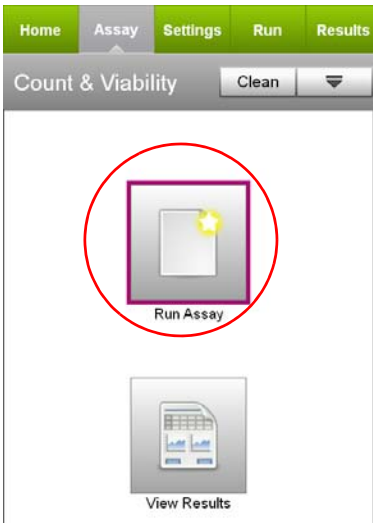
3. Allow the cells to stain for a minimum of 5 minutes.

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 **Count & Viability** from the main menu.

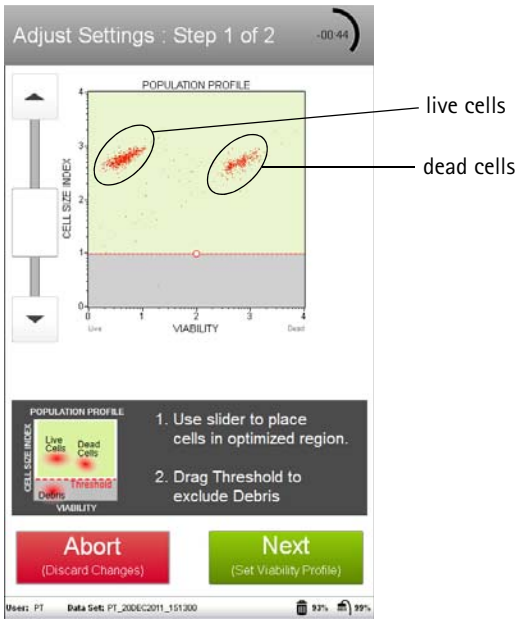
2. Select **Run Assay**.



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*.

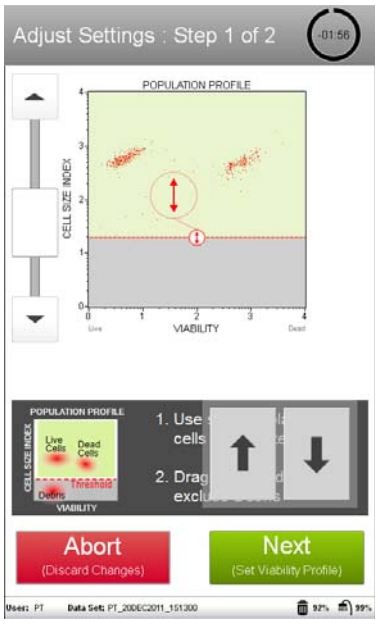
4. Fine tune the settings for the VIABILITY vs CELL SIZE INDEX plot, if necessary.
   - Adjust the Cell Size Index slider to the left of the plot to move the cellular population into the green region.
   - Drag the threshold to exclude any cellular debris. Touch the threshold and drag to make large changes. Touch the arrow buttons located below the plot to make small changes. The arrow buttons appear after you touch the threshold.

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.



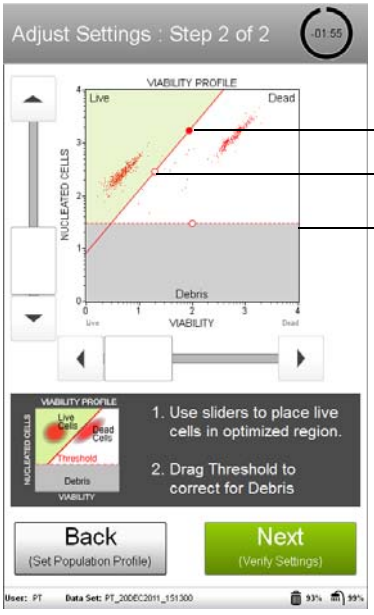
live cells

dead cells



This example data show typical gate and marker settings. The threshold was raised to remove debris.

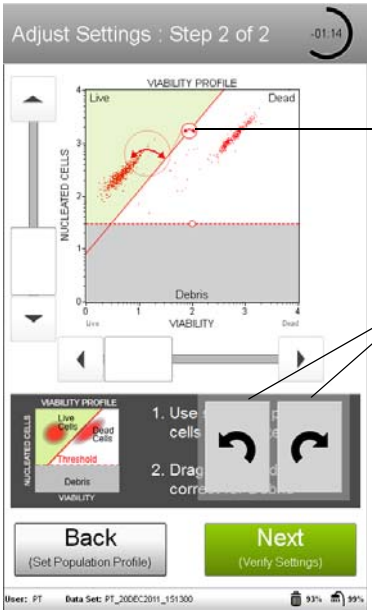
5. Select **Next** when you've completed the adjustments.
6. Fine tune the settings for the VIABILITY vs NUCLEATED CELLS plot, if necessary.
   - Adjust the vertical and horizontal sliders (to the left of and below the plot) to position the viable cells in the upper left (green region) and the dead cells in the upper right (white region) of the plot.
   - Adjust the horizontal line to separate the viable cells from debris. Be sure to exclude all debris.
   - Adjust the vertical/angled marker (viability discriminator) to separate the viable cells (left) from the dead cells (right). You can move the marker from left to right, as well as adjust the angle. To move from left to right, touch the open circle and drag the line, or touch the arrow buttons below the plot. To adjust the angle, touch the solid circle and drag in an arc, or touch the arrow buttons below the plot.



Touch to adjust angle.

Touch to move right/left.

Touch to move up/ down.

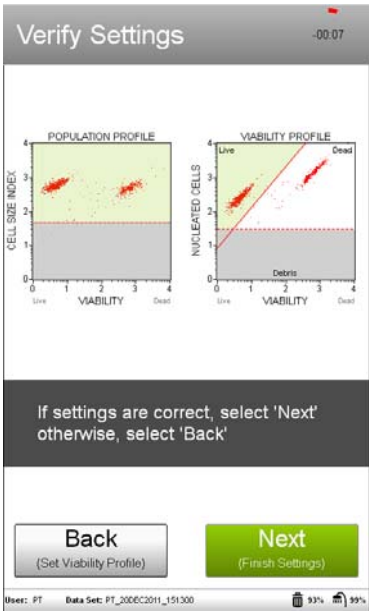


Drag to adjust the angle.

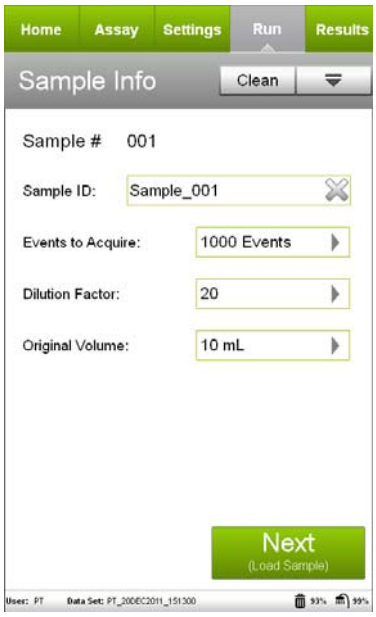
Or, touch to adjust the angle.

7. Select **Next** when the 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.



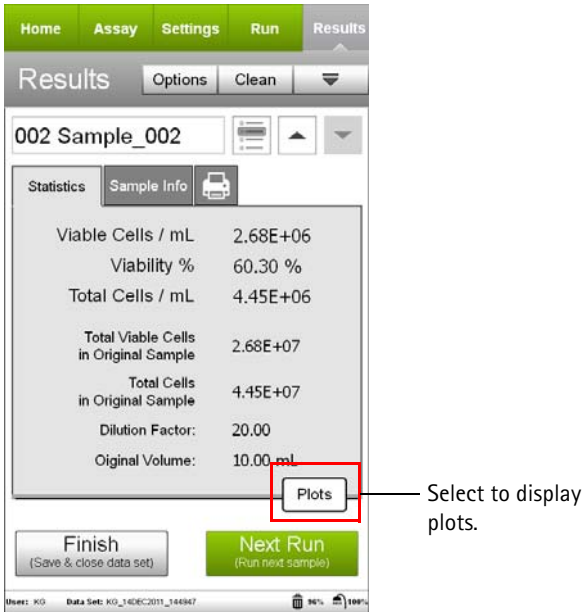
9. Enter the sample ID by touching the field, then using the keypad to input the ID. Touch Done when you've finished entering the ID. If necessary, change the Events to Acquire, Dilution Factor, and/or Original Volume by touching the field, then selecting the value from the pop-up menu. Select **Next**.

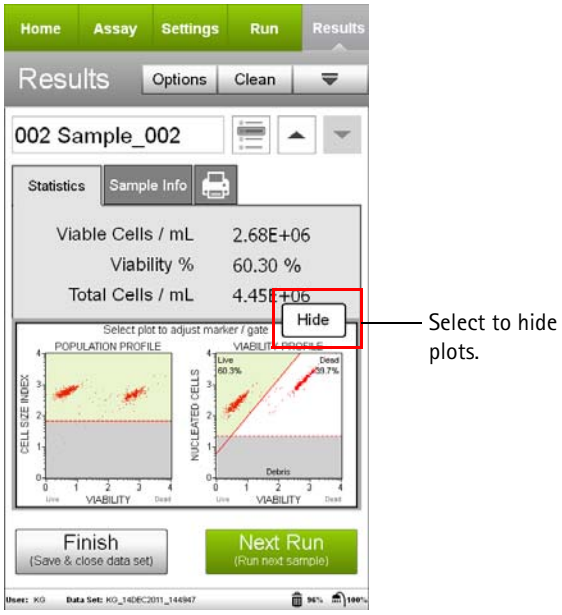


10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.

11. When acquisition is complete, the results are displayed. If necessary, select **Plots** to display dot plots for the sample.

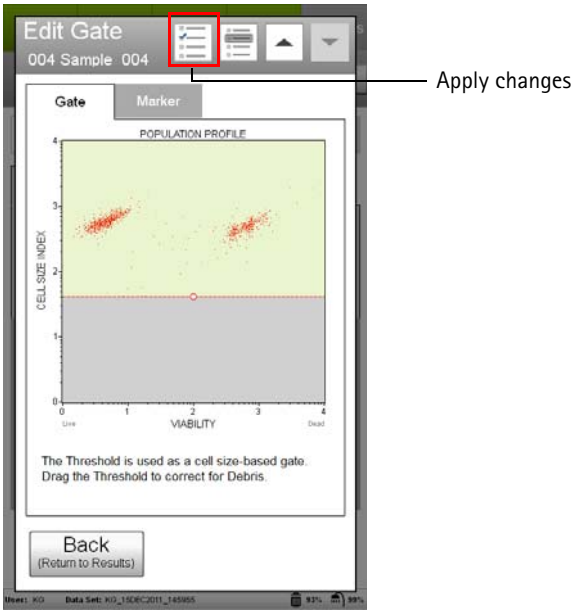
    You can view or change the sample ID, dilution factor, and original volume, as well as add annotations for the current sample by selecting the Sample Info tab. To print the results for the current sample select the printer tab.

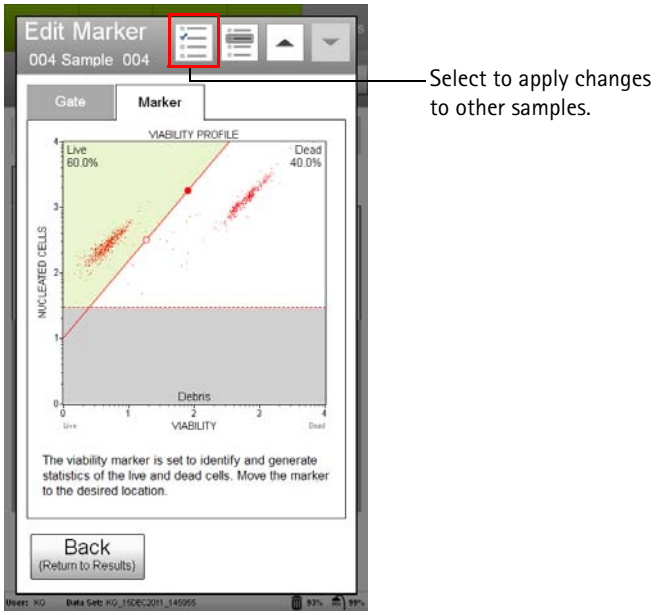




12. (Optional) If changes are needed to the cell size gate or viability marker, touch a plot to enlarge it, then adjust the cell size gate and/or viability marker as described in steps 4 and 6. You cannot adjust the nucleated cells threshold after the sample has been acquired.

    If you adjust the gate or marker 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.





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

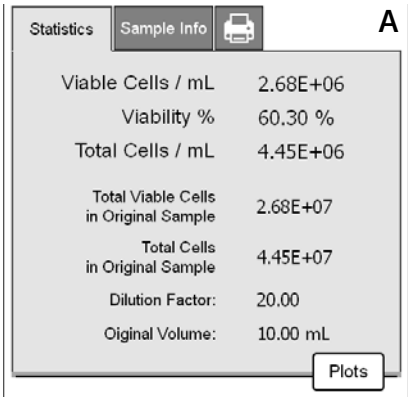
    NOTE: During the run, a message may appear prompting you to load a tube of DI water for a Quick Clean. Load the water then select **Clean** to perform the Quick Clean. Select **Next** to continue with the run. The frequency of Quick Cleans was set by your system administrator. Your administrator may 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.

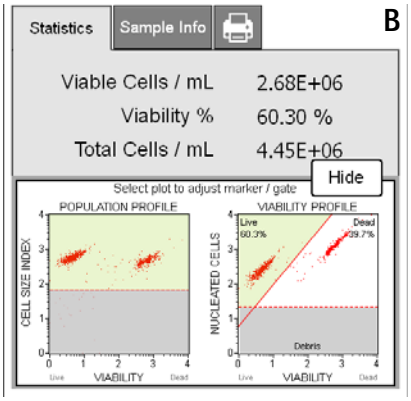
14. When you have acquired the last sample, select **Finish**.
15. (Optional) Select **Options** in the title bar to rename the data set, export the data set, save the current instrument settings, or view the event log. Refer to the *Muse™ Cell Analyzer User's Guide* for more information.

Results

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

- sample number
- sample ID
- viable cell concentration (cells/mL)
- percent viability
- total cells per mL
- total viable cells in original sample
- total cells in original sample
- dilution factor (input value)
- original volume (input value)
- fluorescence intensity values for the viable and dead populations





Figures A and B. Healthy Jurkat cells were mixed with heat-killed Jurkat cells and stained with Muse™ Count & Viability Reagent, and then acquired on the Muse™ Cell Analyzer. Figure A shows summary data, while Figure B shows results displayed with optional dot plots. The statistics show the Viable Cells/mL, the % Viability, and the Total Cells/mL for the Jurkat sample shown. The first plot in Figure B shows Viability vs Cell Size; the second plot shows Viability vs Nucleated Cells.

Technical Tips

1. Mix each cell sample thoroughly on a mixer before acquiring samples for consistent and accurate results. However, avoid vigorous mixing, which can cause cellular breakdown and splashing, resulting in volume loss and erroneous results.
2. Multiple acquisitions of a cell sample minimizes sampling error. Statistically, multiple acquisitions yield more accurate cell counts and viability results.
3. The default number of events to acquire is 1000. You may select a different number; however, your statistical error will increase as you decrease the number of acquisition events.
4. If results deviate from expected values, prepare a freshly stained sample and reacquire the data.
5. If the cell count results deviate from expected values, check that the correct values were entered for dilution factor and original volume of the cell suspension. The Count & Viability application can be used to recalculate cell counts. Open the data file corresponding to the mistaken entry. Reenter the correct dilution factor or original volume and the cell count values will be recalculated automatically.
6. Periodically run Quick Clean using a tube of DI water (after every 20 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.
7. 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 100 µL of sample in the tube. If not, repeat the sample preparation or proceed to the next sample. If the sample volume is greater than 100 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 Quick Clean 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 Corporation's Technical Support for help.
8. Count & Viability works best with samples in a homogeneous, single cell suspension. Cell aggregates may clog or be excluded from the flow cell, affecting the accuracy of your results. If you want to use the Muse™ Count & Viability assay with a "clumpy" cell line, such as Chinese Hamster Ovary (CHO) cells, we recommend that you order Muse™ Cell Dispersal Reagent (Catalog No. MCH100107) to disaggregate the cells. Contact Customer Service or visit our website at www.millipore.com/muse for detailed information on the Muse™ Cell Dispersal Reagent and assay method. For more troubleshooting tips, refer to the *Muse™ Cell Analyzer User's Guide*.

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.
Instrument clogging; too many cells	Run a Quick Clean to clean out capillary. This procedure can be performed during or after an assay. This will wash away any material forming within the glass capillary walls.
Low Cell Concentration warning during acquisition	The sample concentration may be too low. The assay instructions are optimized to give you a range of cells between 100–500 cells/µL in the final sample volume so accurate population count results are obtained. Repeat sample preparation with a lower dilution factor to allow for adequate cell numbers. 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 for acquisition is high (>500 cells/µL), the accuracy of data will most likely be compromised. Repeat sample preparation with a higher dilution factor to allow for adequate cell numbers.
Background staining and/or non-specific staining of cells	If cells have high background staining, the cells may be damaged, as dead cells tend to aggregate and non-specifically adsorb fluorescent reagent. Avoid damaging cells when handling them in culture.
Low level of staining	Although the assay procedure has been optimized to function utilizing multiple cell types, every cell line behaves differently. A lack of signal may indicate that excess dilution factors may need to be altered to obtain accurate results. Ensure proper controls are used.
Variability in day-to-day experiments	• 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. Run the Muse™ System Check procedure (Catalog No. MCH100101) to verify proper instrument function and accuracy. • Always monitor threshold settings, especially if using different cell types, to ensure cell events are not excluded.

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.

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 x 10^5 to 1 x 10^7 cells/mL in serum- or albumin containing medium.
2. Proceed to "Staining Protocol" 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 so final concentration is between 1 x 10^5 to 1 x 10^7 cells/mL.
3. Proceed to "Staining Protocol" on page 4.

References

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