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Muse® Count & Viability Kit (200X) User's Guide

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Application

The Muse® Count & Viability Reagent (200X) was developed for absolute cell count and viability determination of difficult cell samples. It is designed to address the need for a compatible reagent for non-mammalian cell lines, such as SF-9 insect cells. These cells prefer significantly lower pH and higher osmolarity than mammalian cells for optimal viability. The reagent contains the same fluorescent DNA-binding dyes used in Muse Count & Viability Reagent, but in a highly concentrated formulation. These dyes have differential permeability to viable and non-viable cells, and providing absolute cell count and viability data on cell suspensions from a variety of cultured 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 using the Guava® Muse Cell Analyzer with the Muse® software provides:

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

Muse Count & Viability Reagent (200X) allows you to assay a greater variety of cell types on the Guava Muse Cell Analyzer. You can use Count & Viability Reagent (200X) to prepare a custom formulation of reagent in a buffer or medium compatible with your special cell line. Count & Viability Reagent (200X) can be added directly to the cell sample for counting. This allows direct and rapid determination of absolute cell count and viability with dilute cell samples, down to 1×10^4 cells/mL.

Muse Count & Viability Reagent (200X) can be used to prepare a more concentrated, custom formulation of Muse Count & Viability Reagent, which may reduce incubation time and optimize fluorescent signals for cell lines that stain less efficiently. Count & Viability Reagent (200X) allows assessment of absolute cell count and viability of challenging cell samples rapidly, reliably, and conveniently.

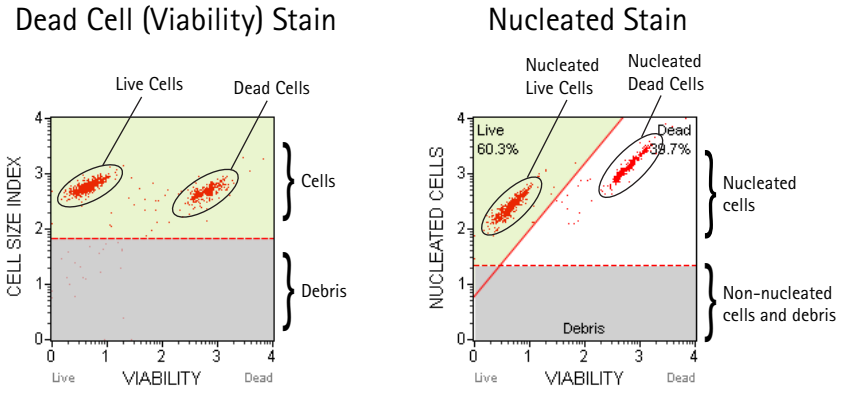
Sufficient reagent is provided for the preparation and analysis of 100 tests.

Test Principle

The Muse® Count & Viability Reagent (200X) 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.



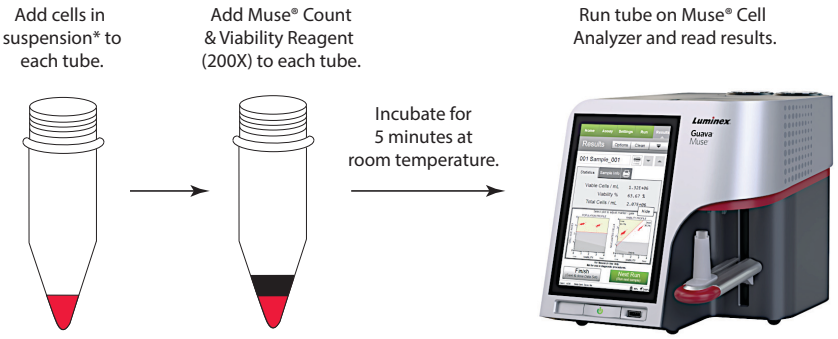
	Debris and Non nucleated cells	Viable Cells	Dying Cells	Dead Cells
Cell Size Index	Low	High	Mid to High	High
Nucleated Cell Stain	Neg	High	High	High
Viability Stain	Neg	Neg	Med	High

Nucleated cell stain differentiates cells from debris.

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

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 Kit (200X) [Catalog No. MCH100104]

- Muse® Count & Viability Reagent (200X) [Part No. 4000-0325, 0.2 mL]

Materials Required but Not Supplied

- Guava® Muse® Cell Analyzer
- Cell suspension
- Dilution buffer: complete growth media or phosphate buffer solution appropriate for your cell culture
- Capped tubes or bottles for preparing reagent solutions
- Serological pipets, sterile
- 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
- Guava® ICF Instrument Cleaning Fluid (Catalog No. 4200-0140), optional
- Muse® System Check Kit (Catalog No. MCH100101), optional

Precautions

- The Muse® Count & Viability Reagent (200X) is intended for research use only.
- The Muse Count & Viability Reagent (200X) 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 SDS for specific information on hazardous materials.
- The Muse Count & Viability Reagent (200X) 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.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
- 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.
- Safety Data Sheets (SDS) for kit reagents are available from our website (*www.luminexcorp.com*), by contacting Luminex Technical Support.

Storage

- Store the Muse® Count & Viability Reagent (200X) frozen at –15 to –25°C for long-term storage. Refer to the expiration date on the package label. We do not recommend using the reagent past the expiration date.
- Count & Viability Reagent (200X) contains light-sensitive dyes. Shield from excessive exposure to light.
- Muse Count & Viability Reagent (200X) and concentrated dilutions of the reagent may be refrozen at –15 to –25°C for storage. Count & Viability Reagent (200X) may be repeatedly thawed and refrozen up to 10 times.

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 1×10^4 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 (200X), the stability of individual cell types may vary.
Optimal staining times and fluorescent signals can vary from cell line to cell line with Count & Viability Reagent (200X). For example, stained Jurkat (human T-lymphocytic leukemia) cells exhibit higher mean fluorescence intensities than HL60 (human promyelocytic leukemia) cells when incubated with Count & Viability Reagent (200X) for 5 minutes. Some cell lines, such as SF-9 insect cells, exhibit incompatibility when incubated with Count & Viability Reagent (200X) for extended periods of time. Although counting and viability results are not usually affected by this variation, optimal fluorescent signals can make analysis easier and more consistent.
Time considerations: The cell staining procedure with the Muse Count & Viability Reagent (200X) takes 5 minutes. Acquiring data on your Guava® 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*.

Assay Considerations

The concentration and number of the cell samples for analysis will dictate the appropriate choice of reagent and sample preparation methods. If your cell sample is at a low to moderate density (1×10^4 to 5×10^5 cells/mL), Muse® Count & Viability Reagent (200X) should be added directly to the cell suspension. If your cell sample is from a cell line that is incompatible with the standard Muse Count & Viability Reagent (eg, SF-9, HL60 cells), and is at a moderate to high density (1×10^5 to 1×10^7 cells/mL or higher), the cell sample must be diluted with culture medium or a compatible buffer to a concentration of 1×10^4 to 5×10^5 cells/mL. See Table 1 for recommended preparation volumes per test. If you have a large number of these types of samples to assay at one time, it will be

more convenient to prepare a custom diluted Count & Viability Reagent formulation using compatible buffer or culture medium, and perform the assay as described in "Staining Protocol."

Table 1: Sample Preparation with Count & Viability or diluted in buffer/medium (per test sample)

Conc. of Suspension (cells/mL)	Cell Dilution Factor	Count & Viability Volume Stock Solution	Medium/Buffer Volume	Cell Volume
1×10^4 to 5×10^5	1	2 µL	0 µL	400 µL
1×10^5 to 1×10^6	10	2 µL	180 µL	20 µL
1×10^6 to 1×10^7	20	2 µL	380 µL	20 µL
1×10^7 to 2×10^7	40*	2 µL	780 µL	20 µL
*Further dilution will be required for samples at cell densities >2×10^7 cells/mL.				

Diluting the Reagent

IMPORTANT NOTE: Thaw the Count & Viability Reagent (200X) completely and mix well before use.

Prepare only enough diluted Count & Viability Reagent (200X) for immediate use to avoid problems with microbial contamination. The reagent may not be sterile. Do not freeze the diluted reagent formulation. Discard any unused diluted reagent.

1. Add the appropriate amount of Count Count & Viability Reagent (200X) to a 1.5-mL tube.
2. Add the appropriate amount of culture medium or buffer.
3. Mix well until the reagent is fully dissolved. There should be no dark purple reagent visible at the bottom of the tube, and the solution should appear pink.

Staining Protocol

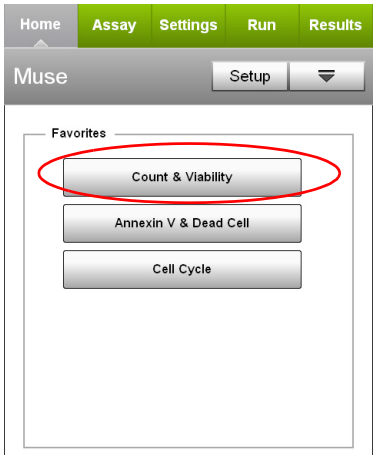
Low Concentration Samples: Use Muse® Count & Viability Reagent (200X) stock solution.
Moderate to High Concentration Samples: Prepare the reagent by diluting Count & Viability Reagent (200X) 1:200 with cell culture medium or compatible buffer for your cell line, as described in Table 1.

1. Mix the cellular sample well by vortexing or repeated pipetting.
2. Transfer the appropriate volume of the cell suspension to a 1.5-mL microcentrifuge tube, as described in Table 1.
3. Add the appropriate volume of the Count & Viability Reagent (either diluted or stock, as determined above) to the sample tube. Mix well until the solution appears homogeneously pink in color. There should be no dark purple reagent visible at the bottom of the tube.
4. Incubate the sample for a minimum of 5 minutes at room temperature to allow dye staining to equilibrate.
5. The sample is ready for data acquisition on the Guava® Muse Cell Analyzer using the Count & Viability software module. Mix the cell sample well before loading it onto the instrument.

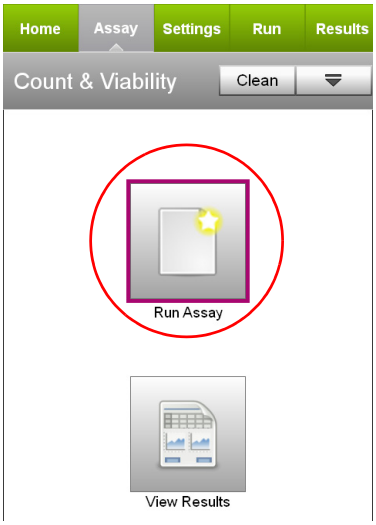
Setup and Acquisition on the Guava® Muse® Cell Analyzer

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

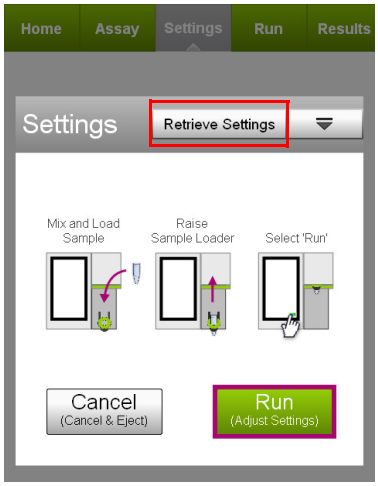
1. Select **Count & Viability** from the main menu.



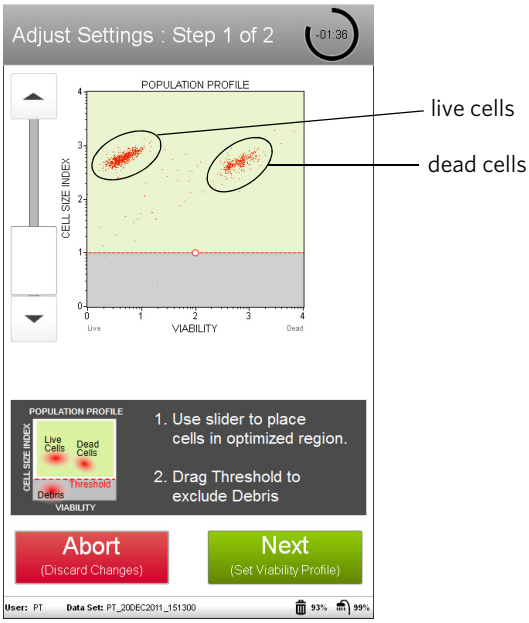
2. Select **Run Assay**.

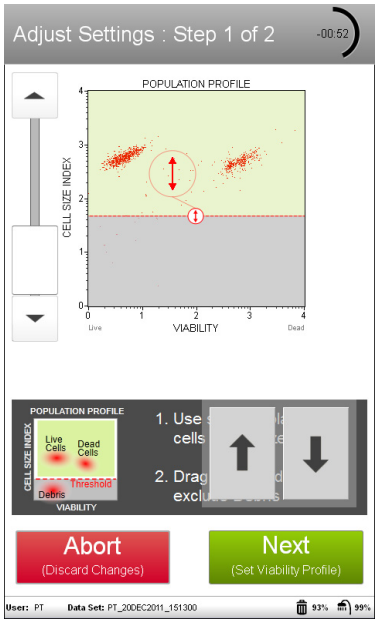


3. Adjust the instrument settings.
   - Load a stained sample for adjusting the settings and select **Run**.
   - Or, to retrieve previously saved instrument settings, select **Retrieve Settings**. For more information on retrieving settings, see the *Muse Cell Analyzer User's Guide*.
4. Fine tune the 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 marker to exclude any cellular debris. Touch the threshold and drag to make large changes. Use 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.

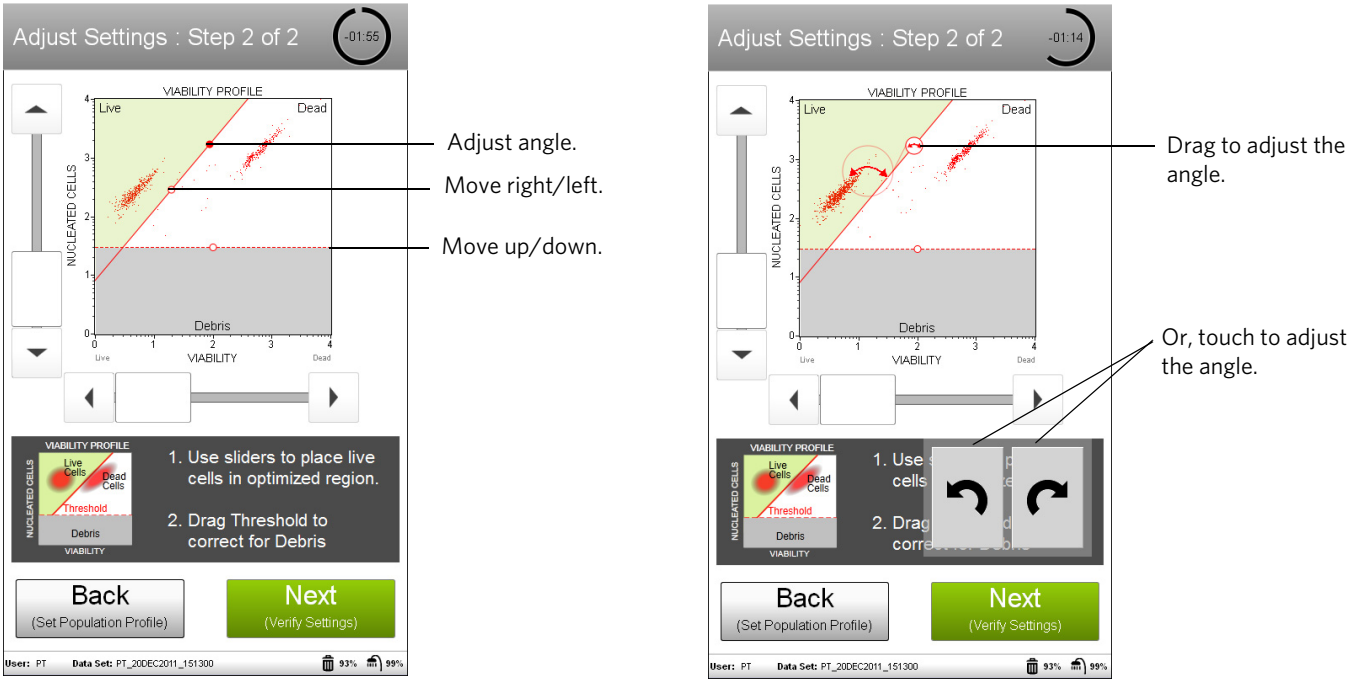




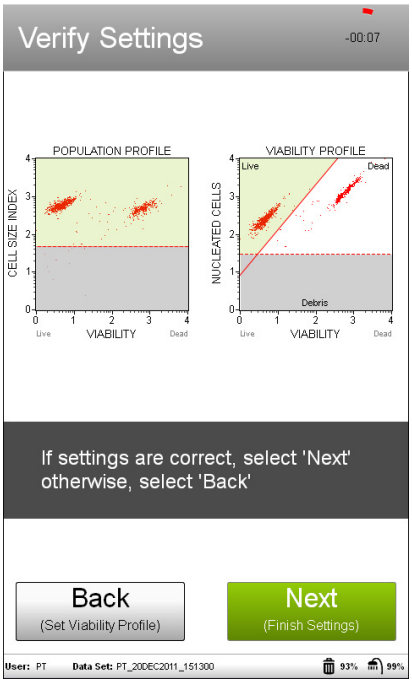
This example data show typical gate and marker settings. The threshold was raised to remove debris.

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

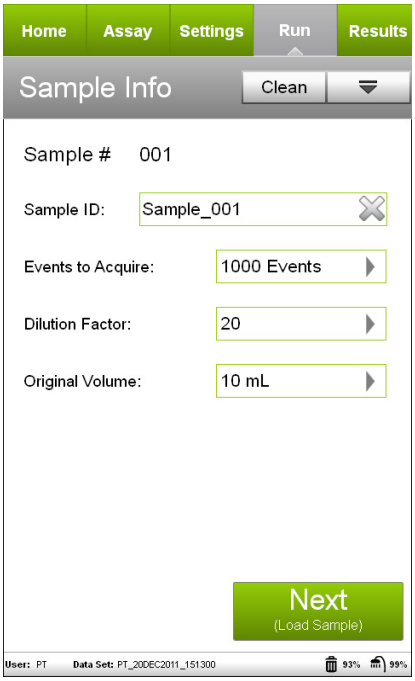
right, touch the open circle and drag the line, or touch the arrow buttons below the plot. To adjust the angle, touch the solid circle and drag in an arc, or touch the arrow buttons below the plot.



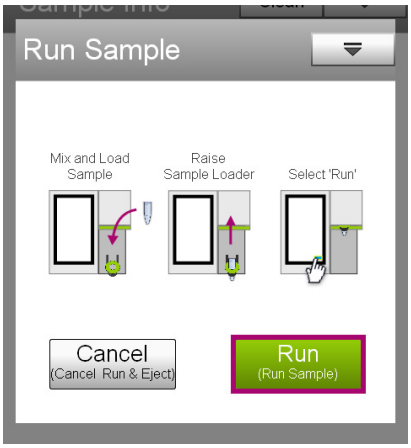
7. Select **Next** when the adjustments are complete.
8. Verify the settings. If the settings are correct, select **Next**. Otherwise, select **Back** and repeat steps 4 through 7, as necessary.



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**.

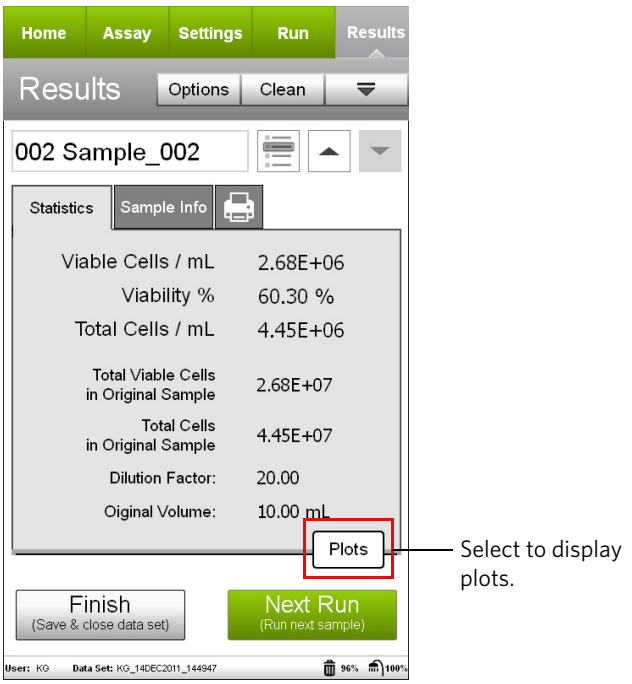


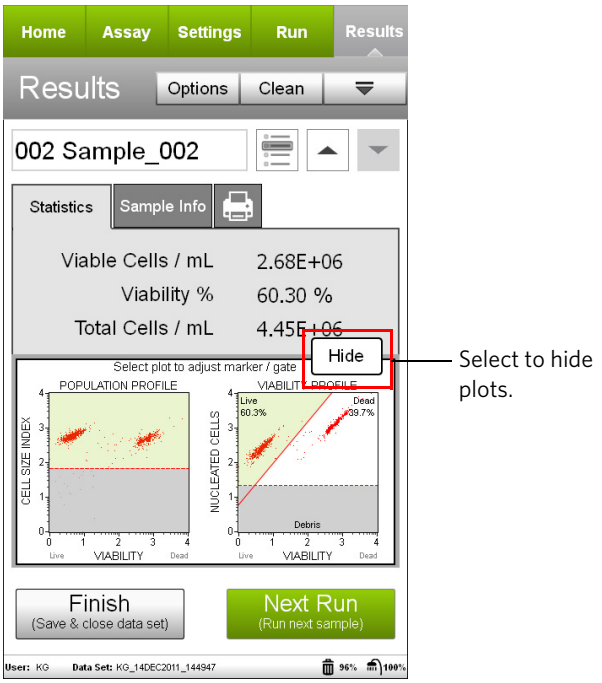
10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.



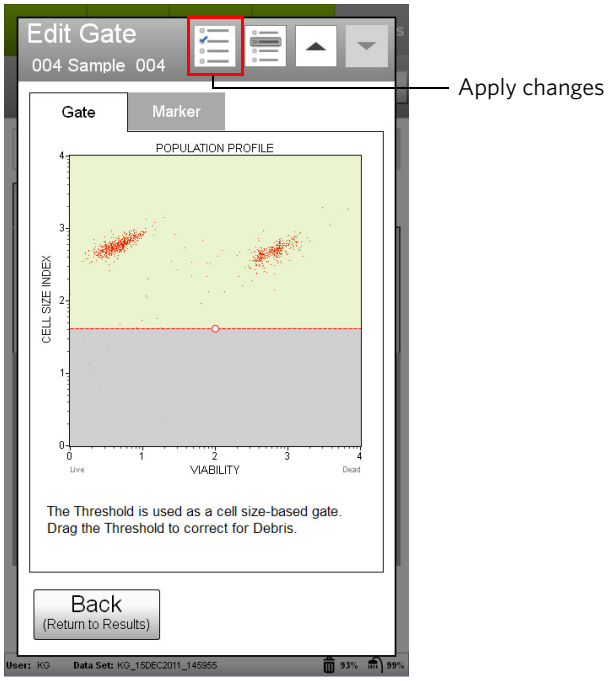
11. When acquisition is complete, the results are displayed. If necessary, select **Plots** to display dot plots for the sample.

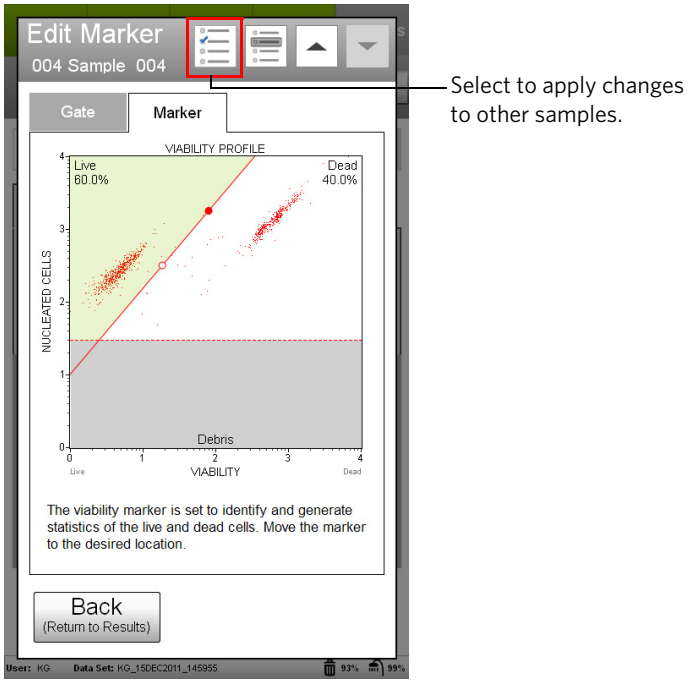
You can view or change the sample ID, dilution factor, and original volume, as well as add annotations for the current sample by selecting the Sample Info tab. To print the results for the current sample select the printer tab.





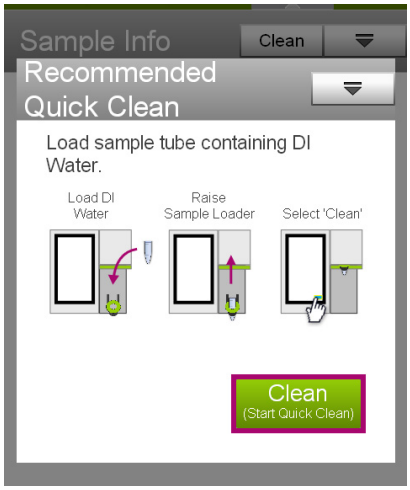
12. (Optional) If changes are needed to the cell size gate or viability marker, touch a plot to enlarge it, then adjust the cell size gate and/or viability marker as described in steps 4 and 6. You cannot adjust the nucleated cells threshold after the sample has been acquired.
    If you adjust the gate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

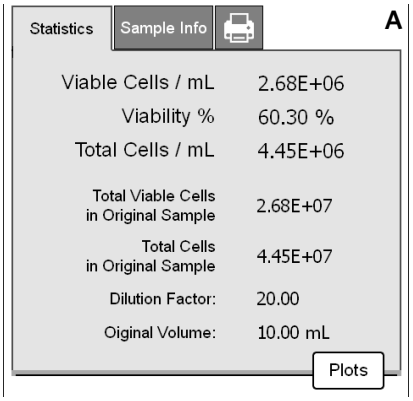


14. When you have acquired the last sample, select **Finish**.
15. (Optional) Select **Options** in the title bar to rename the data set, export the data set, save the current instrument settings, or view the event log. Refer to the *Muse Cell Analyzer User's Guide* for more information.

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



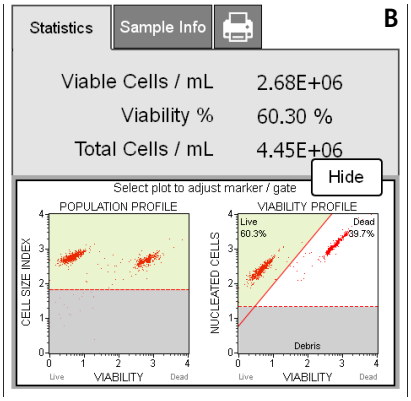


Figure 1: Healthy SF-9 cells were mixed with heat-killed SF-9 cells and stained with Muse® Count & Viability Reagent (200X), and then acquired on the Guava® Muse Cell Analyzer. Figure A shows summary data, while

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 SF-9 sample shown. The first plot in Figure B shows Viability vs. Cell Size; the second plot shows Viability vs. Nucleated Cells.

Technical Tips

1. Careful, accurate pipetting with calibrated pipettors is required for accurate and consistent results in the Count & Viability assay. Mix cell samples well. Cells will settle to the bottom of the tube over short periods of time,
2. Thaw the Count & Viability Reagent (200X) and derivative formulations completely and mix well before using.
3. To avoid bacterial contamination problems, prepare only enough Count & Viability Reagent (200X) diluted in medium or buffer for immediate use. This reagent cannot be sterilized by filtration, because the Count & Viability Reagent dyes will bind to the filter membrane. Autoclaving may be deleterious to the formulation. Addition of preservatives is not recommended without testing for cell sensitivity or toxicity.
4. We recommend acquiring data from test samples as soon as possible after staining incubation is complete. Maintain stained cell samples under conditions favorable to the cells until data acquisition. Leaving samples at room temperature for long periods of time or other suboptimal handling conditions before data acquisition may lead to low viability.
5. Use Guava ICF periodically to clear the fluid system of buildup from cell samples and reagent residue. Run Quick Clean with Guava ICF followed by water after finishing the Count & Viability assay to remove dye residue that could carry over to other assay tubes.
6. Cell samples containing aggregates may not count accurately on the Muse® System. Clumpy cell samples can be disaggregated with Muse Cell Dispersal Reagent (Catalog No. MCH100107), then stained with Count & Viability Reagent for accurate cell count and viability determination. Contact Luminex Corporation Technical Support for information.
7. Cell handling can affect cell staining with the Count & Viability Reagent dyes. When removing adherent cells from the culture substrate using enzymatic treatment (ie, trypsin), take care not to damage the cells by over digestion. If the culture appears to show an unexpectedly low viability, incubate the cell suspension in culture medium for 20 to 30 minutes to allow the cells to recover. Assay a fresh aliquot of the cell suspension with Count & Viability Reagent (200X).

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 Guava® Muse® Cell Analyzer is working properly. Run the Muse System Check Procedure (Part 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 Luminex Technical Support.

Limitations

1. The results of the assay are dependent upon proper use of reagents, products, and instruments.
2. The Count & Viability (200X) assay may not assess the % Viability and Total Cell Count of low viability cell suspensions as accurately as for higher viability samples. In general, the actual viability of cell suspensions with <30% live cells may be lower than reported by the Count & Viability software module. This is due to

changes in cell structure and integrity affecting the staining properties of dead and dying cells. Excessive debris may also interfere with accurate counts.

3. The Guava® Muse® Cell Analyzer and Count & Viability Reagents (200X) yield optimal results when the stained cell sample for acquisition is between 1×10^4 to 5×10^5 cells/mL. To obtain the most accurate counting results, adjust the concentrations to within the recommended range. The range for optimal counting results may vary for different cell lines. You may need to adjust the cell concentration accordingly.
4. Cell samples containing cells of a size range between approximately 7 to 60 micrometers in diameter will yield the most accurate results on the Guava Muse Cell Analyzer. If your cell samples contain significant amounts of cells outside this size range, contact Luminex Technical Support for more information.
5. Cell samples containing aggregates may not yield accurate cell counts or % Viability results. See Troubleshooting Tips, tip 7.
6. The Count & Viability assay may not work with all cell lines. Certain cell types may not stain efficiently causing incorrect cell counts and/or viability results. Cell lines expressing fluorescent proteins (eg, transfectants expressing GFP, YFP, etc) or products (eg, transfectants expressing non-fluorescent proteins) may yield accurate total cell counts but incorrect viable cell counts. The signal from the expressed transfected fluorescent protein or transfected product may be detected in the VIABILITY parameter. This may cause an inaccurately low viability reading for the culture.
7. Culture conditions can affect cell staining with the Count & Viability Reagent (200X) dyes. We have validated the Count & Viability assay on SF-9 and CHO-K1 cells grown in culture medium containing surfactants; however, unusual additives or medium components and drugs may interact with the reagent or affect the assay.

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, 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.
2. Proceed to “Staining Protocol” on page 5.

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 1×10^5 to 1×10^7 cells/mL.
3. Proceed to “Staining Protocol” on page 5.

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Related Kits

- Muse® System Check Kit – MCH100101
- Muse® Count & Viability Kit (40 mL) – MCH100102
- Muse® Count & Viability Kit (240 mL) – MCH600103
- Muse® Annexin V & Dead Cell Kit – MCH100105
- Muse® Cell Cycle Kit – MCH100106
- Muse® Cell Dispersal Reagent – MCH100107