Luminex®
complexity simplified.

Muse® Cell Dispersal Reagent

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For Research Use Only
4600-3381, Rev D
Catalog No. MCH100107 (100 tests)
June 2019

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Application

The Muse® Cell Dispersal Reagent is an enzymatic reagent that has been formulated to gently disaggregate the cells in aggregates and clumps to obtain uniform suspensions for cell counting. Cell samples containing cell aggregates may not yield accurate and consistent results with Muse® Count & Viability Reagent alone due to differential staining in clumps and the possibility that cell aggregates may clog or result in coincident events, hampering the assay.[1-3] Cell lines such as Chinese Hamster Ovary (CHO-K1) cell lines that have been adapted to suspension culture tend to form cell aggregates.[4,5] Muse Cell Dispersal Reagent is useful for disaggregating clumpy suspension cultures in combination with Muse Count & Viability Reagents, and yields improved accuracy and consistency of count and viability data in the Muse Count & Viability Assay.[2]

Data generated with the Muse software module provides:

- Viable cell count (cells/mL)
- Total cell count (cells/mL)
- % viability of sample

The Muse Cell Dispersal Reagent is for use for treatment of clumpy cells with the Muse Count & Viability Reagent and the Guava® Muse Cell Analyzer. The Guava Muse System makes sophisticated fluorescent based analysis fast, easy, convenient, and affordable. Sample preparation is minimal, and after loading 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 tests.

Test Principle

The Muse® Cell Dispersal Reagent dissociates cell aggregates based on its proteolytic, collagenolytic, and DNAse activity. The reagent can therefore be very effective for cell aggregate dissociation, yielding an improved consistency of cell counts when used in combination with the Muse Count & Viability Reagent.

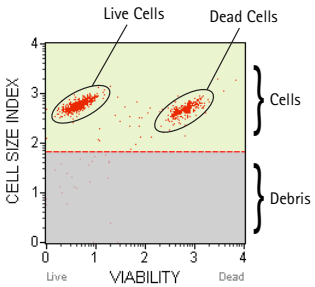
The Muse Count & Viability Reagent differentially stains viable and non-viable cells based on their permeability to the two DNA binding dyes present in the reagent. The Muse Count & Viability software module then performs calculations automatically and displays data in two dot plots:

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).

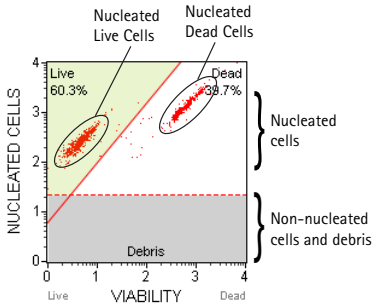
A membrane-permeant DNA staining dye that stains all cells with a nucleus. This parameter is displayed as NUCLEATED CELLS and is used to discriminate cells with a nucleus from debris and non-nucleated cells. The

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).

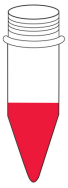
Each plot has moveable markers. The first plot has a gate marker, allowing you to eliminate debris based on size. The second plot also has a threshold marker, allowing you to eliminate cells that do not have a nucleus. This plot also has an angled marker (viability discriminator), allowing you to separate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

Summary of Protocol

Add 50 µL of cell suspension, 50 µL of Muse® CDR working solution,* and 150 µL of Muse® Count & Viability Reagent to each tube.

Incubate for 20 minutes at 37°C.

Add 250 µL of Muse® Count & Viability Reagent to each tube.



Incubate for 5 minutes at room temperature.

Run samples on Muse® Cell Analyzer and read results.

Kit Components

Muse® Cell Dispersal Reagent (Part No. 4100-1790, 5 x 1 mL, 100 tests)

Materials Required by Not Supplied

- Guava® 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® Count & Viability Kit (Catalog No. MCH100102 [40mL] or Catalog No. MCH600103 [240mL])
- Guava® ICF Instrument Cleaning Fluid (Catalog No. 4200-0140), optional
- Muse® System Check Kit (Catalog No. MCH100101)

Precautions

- The Muse® Cell Dispersal Reagent is intended for research use only.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
- The Muse Cell Dispersal Reagent is a sterile solution. 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 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 may 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.

Storage

- Store the Muse® Cell Dispersal Reagent frozen at –20°C. Do not store at room temperature.
- Refer to the expiration date on the package label. Do not use the reagent after the expiration date.
- The Muse Cell Dispersal Reagent can be thawed at 2 to 8° or at room temperature. After thawing, the Muse® Cell Dispersal Reagent may be stored at 2 to 8° and is stable for 1 week. The Muse Cell Dispersal Reagent may be re-frozen once more for longer term storage. Repeated freezing-thawing reduces enzymatic activities and should be avoided.

Before You Begin

This protocol was developed to allow direct determination counts and viability of suspension-culture CHO-K1 cells. The kit will give reliable staining and results with cell concentrations in the range of 2 x 10^5 to 5 x 10^6 cells/

mL. Optimization of this assay involves balancing the efficiency of enzymatic clump disaggregation against the digestive loss of the dead cells. In some cases, cell samples containing many large cell aggregates may require a higher concentration (0.8X to 1X) of Muse® Cell Dispersal Reagent or longer incubation times to effectively break up the aggregates. Lower concentrations of Muse Cell Dissociation reagent (0.2X to 0.8X) or shorter incubation times may yield more accurate results for cell samples with less aggregation or of low viability (<25%). It is highly recommended that cell samples be run within 10 minutes after the final incubation in the Muse® Count & Viability Reagent.

Time considerations: The process of disaggregating and staining cells with the Muse Cell Dispersal Reagent and Muse Count & Viability Reagent, respectively will take up to 30 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*.

Cell Disaggregation and Staining Protocol

1. Thaw the Muse® Cell Dispersal Reagent and allow it to come to room temperature.
2. Prepare fresh Muse Cell Dispersal Reagent working solution according to the following table.

   NOTE: Unused Muse Cell Dispersal stock reagent can be re-frozen once more for storage. Avoid using Muse Cell Dispersal Reagent that has been frozen/thawed more than twice, as the repeated freezing-thawing reduces enzymatic activities.

Table 1: Muse Cell Dispersal Reagent Working Solution Preparation Guidelines

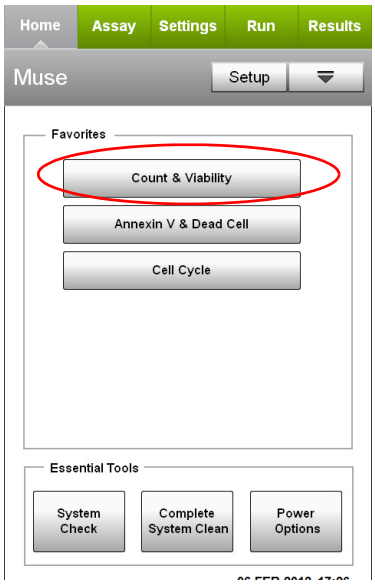
	Muse® CDR working solution (1 test)	Muse® CDR working solution (10 tests)	Muse® CDR working solution (25 tests)
Volume of Muse® Cell Dispersal Reagent	40 µL	400 µL	1 mL
Volume of PBS	10 µL	100 µL	0.25 mL

3. Obtain a well-mixed clumpy cell sample for assay. Cell samples should be at a concentration of 2 x 10^5 to 5 x 10^6 cells/mL for the assay. If your cell samples are more concentrated, dilute them using PBS dilution buffer to bring the cell concentration to this range.
4. Disaggregate and stain the cell samples by mixing 50 µL of cells, 50 µL of Muse® Cell Dispersal Reagent working solution (from step 2), and 150 µL of Muse Count and Viability Reagent in a 1.5-mL microcentrifuge tube. Cap the tube and vortex gently to mix.
5. Place sample tubes in an incubator at 37°C. Incubate tubes for 20 minutes.
6. Add 250 µL of Muse Count & Viability Reagent to each tube and allow the cells to stain for at least 5 minutes at room temperature.
7. Disaggregated cell samples are ready for acquisition. For best results, acquire each sample within 10 minutes after the final incubation with Muse Count & Viability Reagent.

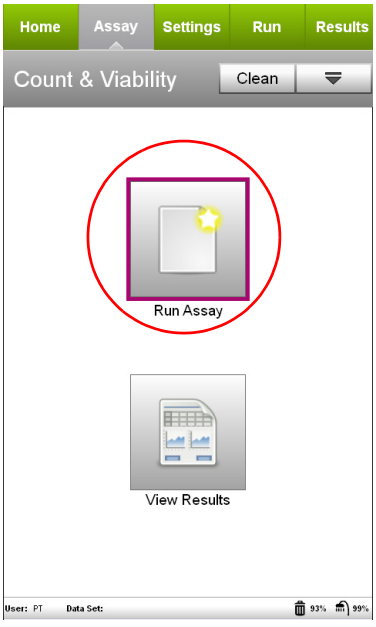
Setup and Acquisition on the Guava® Muse® Cell Analyzer

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

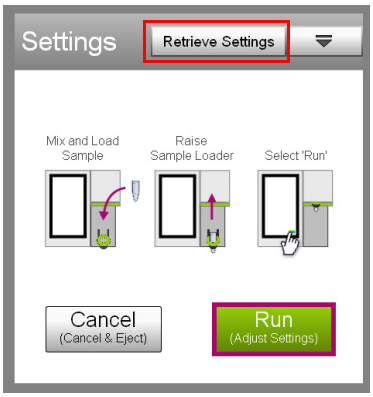
1. Select **Count & Viability** from the main menu.



2. Select **Run Assay**.

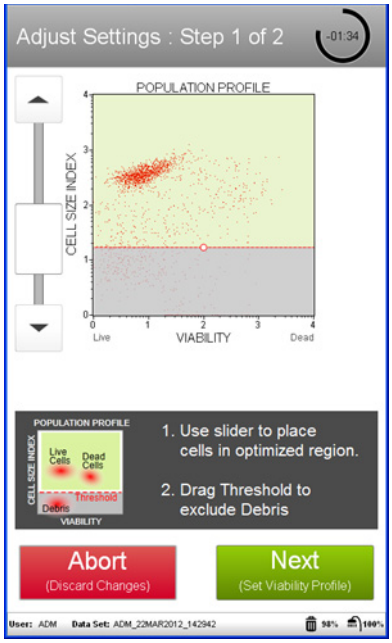


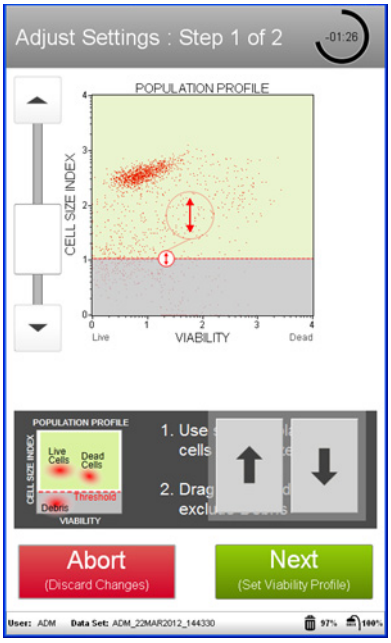
3. Adjust the instrument settings.
   - Load a stained sample for adjusting the settings and select **Run**.
   - Or, to retrieve previously saved instrument settings, select **Retrieve Settings**. For more information on retrieving settings, see the Guava Muse Cell Analyzer User's Guide.



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.

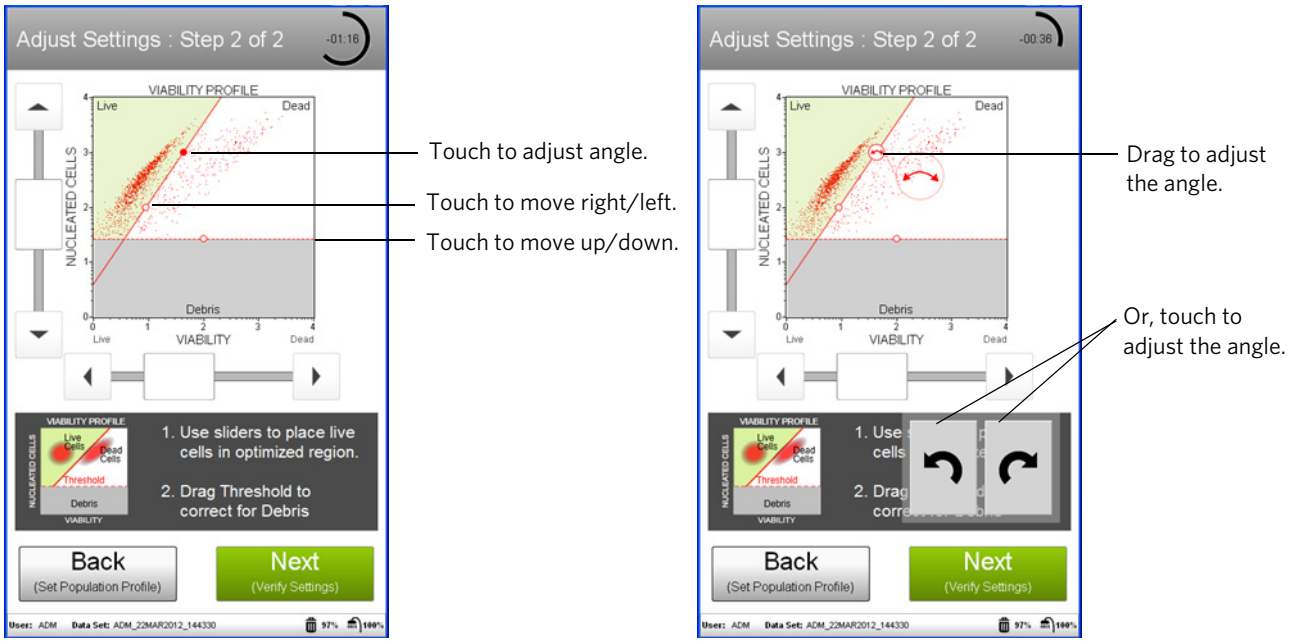
NOTE: If the acquisition times out (after 2 minutes), you can select **Back** to restart the adjust settings step or **Next** to accept the settings and continue to the next step. If acquisition times out, remove the tube and mix well before reloading and continuing.



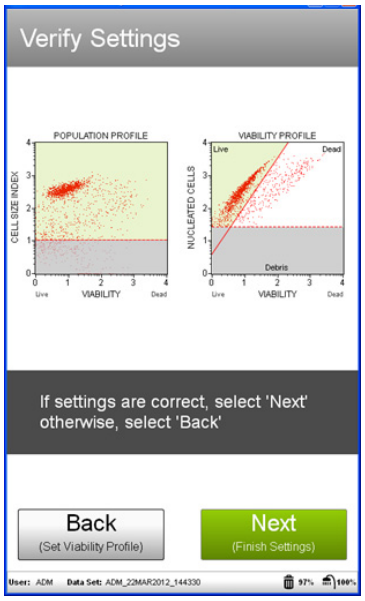


This example data show typical gate and marker settings. The threshold was raised to remove debris.

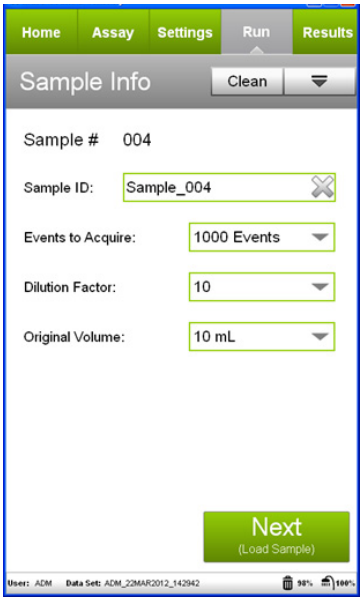
5. Select **Next** when you've completed the adjustments.
6. Fine tune the settings for the VIABILITY vs NUCLEATED CELLS plot, if necessary.
   - Adjust the vertical and horizontal sliders (to the left of and below the plot) to position the viable cells in the upper left (green region) and the dead cells 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.



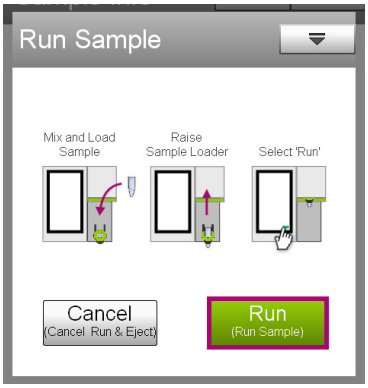
7. Select **Next** when the adjustments are complete.
8. Verify the settings. If the settings are correct, select **Next**. Otherwise, select **Back** and repeat steps 4 through 7, as necessary.



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.

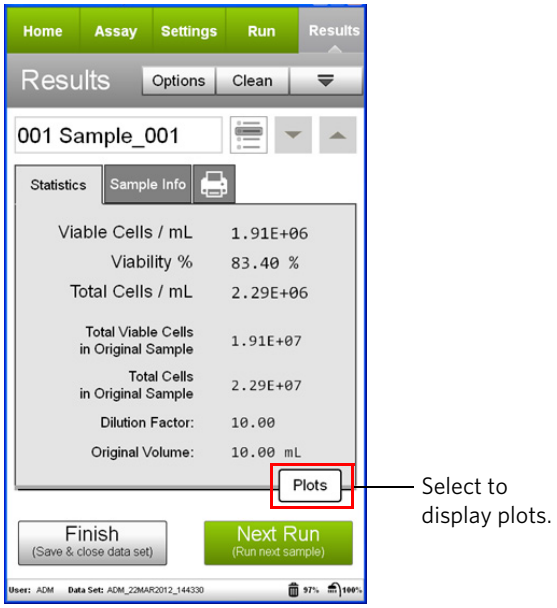


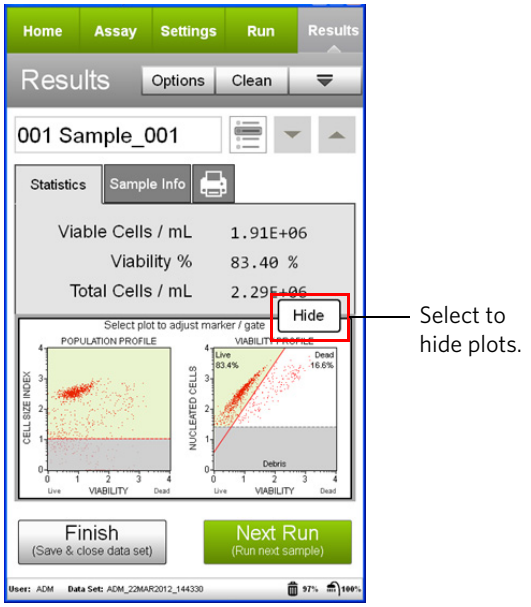
10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.



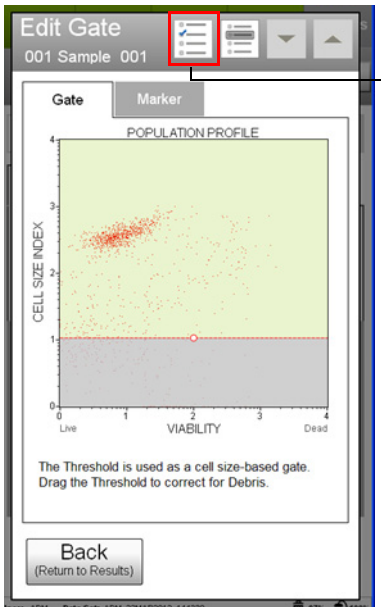
11. When acquisition is complete, the results are displayed. If necessary, select **Plots** to display dot plots for the sample.

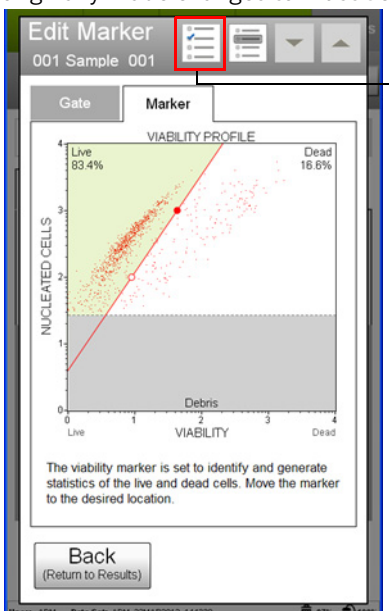
You can view or change the sample ID, dilution factor, and original volume, as well as add annotations for the current sample by selecting the **Sample Info** tab. To print the results for the current sample select the printer tab.





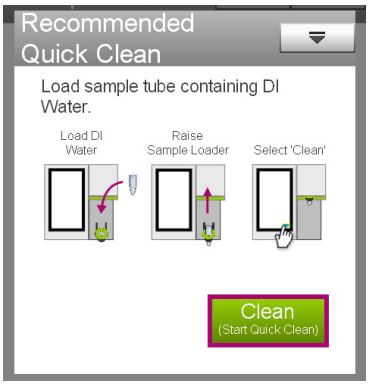
12. (Optional) If changes are needed to the cell size gate or viability marker, touch a plot to enlarge it, then adjust the cell size gate and/or viability marker as described in steps 4 and 6. You cannot adjust the nucleated cells threshold after the sample has been acquired.
    If you adjust the gate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



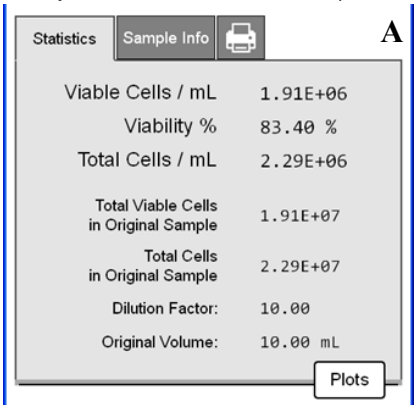
14. When you have acquired the last sample, select **Finish**.
15. (Optional) Select **Options** in the title bar to rename the data set, export the data set, save the current instrument settings, or view the event log. Refer to the Guava Muse Cell Analyzer User's Guide for more information.

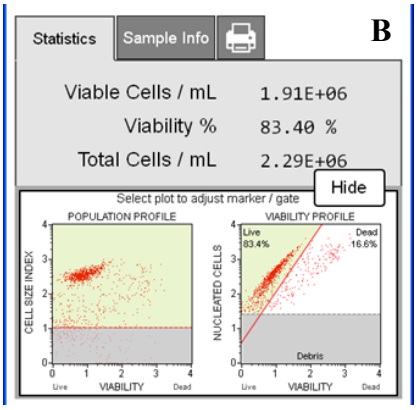
Results

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: Healthy suspension of CHO-K1 cells were disaggregated with Muse® Cell Dispersal Reagent and stained with Muse Count & Viability Reagent, 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 suspension CHO-K1 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 Muse® 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 Guava® Muse Cell Analyzer User's Guide for additional troubleshooting tips, or contact Technical Support for help.
8. Acquire data on disaggregated, stained cells soon after staining. Dilution of the Muse Cell Dispersal Reagent with Muse Count & Viability Reagent slows, but does not stop, enzymatic treatment of the cells. Prolonged exposure of the cells to Muse Cell Dispersal Reagent may result in low total cell counts and high % viability values, due to preferential digestion of dead and dying cells. For best results, acquire the samples within 10 minutes after the final Muse Count & Viability staining step.
9. Fetal bovine serum inhibits Muse Cell Dispersal Reagent at concentrations greater than 5% in the culture medium. If your culture medium contains serum, you must dilute the cell samples with PBS to bring the serum concentration to 5% or lower before using them in the assay.
10. The Muse Cell Dispersal Reagent may not work with all cell lines. Certain cell types may not disaggregate efficiently with the Muse Cell Dispersal Reagent, or stain effectively with the Muse Count & Viability Reagent, causing incorrect cell counts and/or viability results. Cell lines expressing fluorescent proteins (for example, transfectants expressing GFP, YFP, etc.) or other products (for example, transfectant cells lines expressing non-fluorescent proteins) may yield accurate total cell counts but incorrect viable cell counts. Background signal from the expressed transfected fluorescent protein or transfected product may be detected in the Viability parameter.
11. Muse Cell Dispersal Reagent is formulated to meet most suspension CHO-K1 assay requirements. Modification of the assay protocol and reagent concentration(s) may be necessary to ensure optimal performance for individual cell assays.
12. The enzymatic treatment preferentially digests dead cells and dying cells. Optimization of the Muse Cell Dispersal Reagent involves balancing the efficiency of enzymatic clump disaggregation against the digestive loss of the

dead cells. Cells samples containing many large cell aggregates may require a higher concentration of Muse Cell Dispersal Reagent working solution (0.8X to 1X)or a longer incubation time to effectively break up the aggregates. Lower concentrations of Muse Cell Dispersal Reagent working solution (0.2X to 0.8X) or shorter incubation times may yield more accurate results for cell samples with less aggregation or of low viability (<70%). Adding up to 5% fetal bovine serum in the cell sample medium helps to protect dead cells from digestion.

Troubleshooting

Potential Problem	Experimental Suggestion
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 with suspension CHO-K1, 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.
Different viability result observed after cell disaggregation Total Cells/mL result after cell disaggregation is lower than expected	▪ If the viability result after disaggregation is different than expected, the enzymatic treatment might preferentially digest the dead cells over the live, healthy cells, resulting in the skewed result of higher viability. Repeat sample preparation with a higher dilution of Muse® Cell Dispersal Reagent working solution. ▪ If the Total Cells/mL result after disaggregation is lower than expected, repeat sample preparation with a lower dilution of Muse Cell Dispersal Reagent working solution, or increase the incubation time to 30 minutes or longer.

Potential Problem	Experimental Suggestion
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 to verify proper instrument function and accuracy. ▪ Always monitor threshold settings, especially if using different cell types, to ensure cell events are not excluded.

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 2 x 10^5 to 5 x 10^6 cells/mL in low serum- or albumin containing medium.
2. Proceed to *Cell Disaggregation and 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.

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 the final concentration is between 2 x 10^5 to 5 x 10^6 cells/mL.
3. Proceed to *Cell Disaggregation and Staining Protocol* on page 4.

References

1. Muse® Count & Viability User's Guide, Document Part No. 4600-3373. EMD Millipore, 2012.
2. Magallanes E, Helsel C, Liu B, Yokobata K. Validating the Guava® Viacount® CDR™ Assay. Technical Note, Guava Technologies Inc, 2003.
3. Phi-Wilson J, Harvey J, Goix P, O'Neill R. A technology for the rapid acquisition of cell number and viability. *Am Biotechnol Lab*. May 2001:34-36.
4. Shen BP, Bodnar CA, Manuel J, Siegel AC, Levy GA. Comparison of Serum-Free Suspension Culture Media for the Growth and Production of a Protein-Producing CHO Suspension Line. Application Note. Industrial BioDevelopment Laboratory.
5. Hernandez C, Christie A, MacNorton S, Wilson S, et al. Optimization of Cell Counting Throughput for the SAFC Raw Materials Characterization Program. SAFC, Lenexa.

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 Cycle Kit – MCH100106