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Muse® Caspase-3/7 Kit

IMPORTANT: This assay requires that your Guava® Muse® Cell Analyzer has software version 1.3 or later installed for accurate statistical analysis. Check the software version on your Muse® Instrument and update, if necessary. The latest version of Muse® Software can be downloaded from www.luminexcorp.com.

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For Research Use Only
4600-3402, Rev D
Catalog No. MCH100108 (100 tests)
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Application

The Muse® Caspase-3/7 Kit allows for the facile, rapid, and quantitative measurements of two important cell health parameters simultaneously—apoptotic status based on Caspase-3/7 activation, and cellular plasma membrane permeabilization and cell death. The assay provides relative percentage of cells that are live, in the early and late stages of apoptosis, and dead, on both adherent and suspension on the Guava® Muse Cell Analyzer. Minimal sample preparation is required in this no-wash, mix-and-read assay to obtain accurate and precise results. The software provides:

- Percentage of live, early and late apoptotic, total apoptotic, and dead cells.
- Cell Concentration (cells/mL) for live, early apoptotic, late apoptotic, and dead cells

The Muse Caspase-3/7 Kit 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 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

Caspases (cysteinyl-directed aspartate-specific proteases)[1] are cysteine proteases that play a central role in propagating the process of programmed cell death (apoptosis) in response to proapoptotic signals. While some caspases primarily act to initiate intracellular event cascade, other caspases called effector caspases act further downstream and direct cellular breakdown through cleavage of structural proteins (Caspase-3, and Caspase-7). Activation of Caspase-3/7 is thus a hallmark of apoptosis.[3]

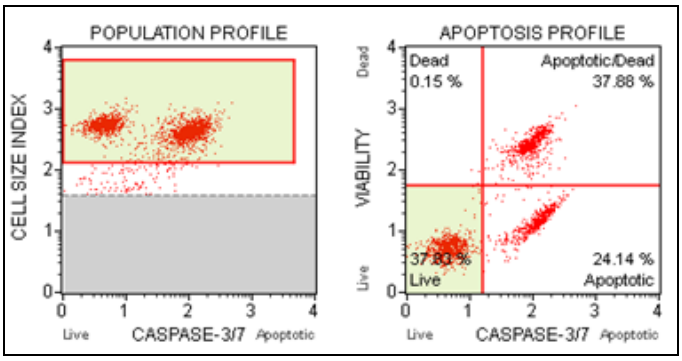
The Muse® Caspase-3/7 Kit simultaneously determines the count and percentage of cells in various stages of apoptosis based on activity of executioner caspases[2] namely, Caspase-3/7 activity in combination with a dead cell dye. The kit utilizes a novel Muse Caspase-3/7 reagent NucView™[4] for the detection of Caspase-3/7 activity and a cell death dye that provides information on membrane integrity or cell death.

- The Muse Caspase-3/7 reagent is cell membrane permeable and non-toxic to the cell. The Muse® Caspase-3/7 reagent contains a DNA binding dye that is linked to a DEVD peptide substrate. When bound to DEVD the dye is unable to bind DNA. Cleavage by active Caspase-3/7 in the cell results in release of the dye, translocation to the nucleus and binding of the dye to DNA and high fluorescence.[4-6] Information on presence of active Caspase-3/7 in the cell is easily obtained when increase in fluorescence in the Caspase-3/7 parameter is observed.
- A dead cell marker (7-AAD) is also included in the assay as an indicator of cell membrane structural integrity and cell death. It is excluded from live, healthy cells, as well as early apoptotic cells. 7-AAD is excluded from live (healthy) and early apoptotic cells, but permeates later stage apoptotic and dead cells. Dead cells thus show increased fluorescence in the Viability Axis.

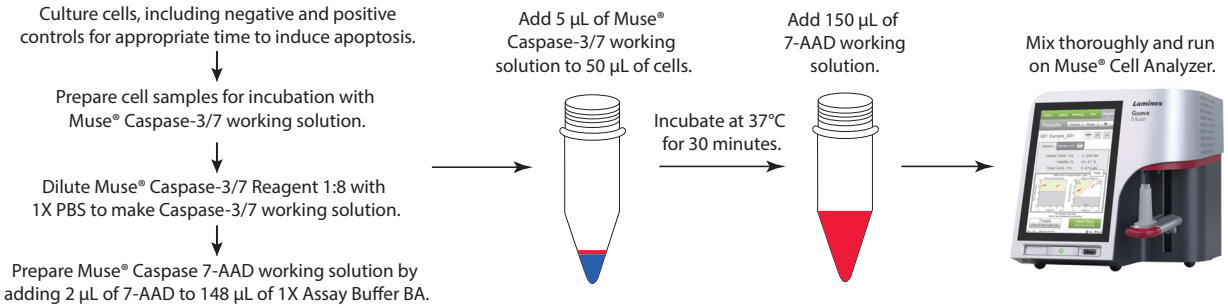
Four populations of cells can be distinguished:

- (LL) Live cells: Caspase-3/7(–) and 7-AAD(–)
- (LR) Apoptotic cells exhibiting Caspase-3/7 activity: Caspase-3/7 (+) and 7-AAD(–)
- (UR) Late Apoptotic/Dead cells: Caspase-3/7(+) and 7-AAD(+)
- (UL) Necrotic cells: Caspase-3/7(–) and 7-AAD(+)

Figure 1: Representative plots from the Muse® Caspase-3/7 Assay. The first plot has a threshold marker, allowing you to eliminate debris based on cell size, as well as a gate to include cells. The second plot shows the gated cells with quadrant marker providing data on four cell populations—Live, Apoptotic, Apoptotic/Dead, and Dead cells.



Summary of Protocol



Kit Components

- Muse® Caspase-3/7 Reagent (Part No. 4700-1505, 100 tests/bottle) [contains NucView™ Caspase-3/7 substrate]
- Muse® Caspase 7-AAD (Part No. 4700-1510, 100 tests/bottle)
- 1X Assay Buffer BA (Part No. 4700-1360, 100 tests/bottle)
- 1X PBS (Part No. 4700-1515, 100 tests/bottle)

Materials Required but Not Supplied

- Guava® Muse® Cell Analyzer
- Cell suspension treated and untreated to undergo apoptosis
- Micropipettors
- Disposable micropipettor tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog No. 16466-030, or equivalent)

- Muse® Count & Viability Kit (Catalog No. MCH100102 (100T) or Catalog No. MCH600103 (600T)), optional
- Vortex mixer
- Disposable gloves
- 20% bleach solution
- Guava ICF instrument cleaning fluid (Catalog No. 4200-0140), optional
- Deionized water
- Muse® System Check Kit (Catalog No. MCH100101)

Precautions

- The Muse® Caspase-3/7 Kit is intended for research use only.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
- The Muse Caspase-3/7 Reagent and Muse® Caspase 7-AAD contain 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 1X Assay Buffer BA 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.
- The 1X PBS is a sterile solution. Exercise aseptic techniques when handling to avoid microbial contamination, 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.
- 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.
- During storage and shipment, small volumes of product may become entrapped in the seal of the product vial. For maximum recovery of product, centrifuge the vial briefly prior to removing the cap.
- Do not use reagents beyond their expiration date.

Storage

- Store the Muse® Caspase-3/7 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 Caspase-3/7 Reagent and Muse® Caspase 7-AAD contain light-sensitive dyes. Shield from excessive exposure to light.

Before You Begin

We 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 3 hours after staining with the Muse® Caspase-3/7 Reagent, the stability of individual cell types may vary.

Time considerations: The process of staining cells with the Muse® Caspase-3/7 Reagent and the recommended protocol will take 30 to 45 minutes. Acquiring data on your Guava® Muse Cell Analyzer takes less than 3 minutes per sample depending on the cell concentration and desired number of events to acquire. However, preparing cells for testing may require periodic maintenance and cultivation. Once you cultivate the proper number of cells for your experiment, it takes an additional 2 to 48 hours of culture with various inducers to stimulate detectable apoptosis.

NOTE: For details on how to culture and prepare cell samples, including positive and negative control samples, see *Appendix A: Cell Sample Preparation* on page 15.

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

Reagent Preparation

Muse® Caspase-3/7 Reagent Working Solution

1. Prepare the Muse® Caspase-3/7 Reagent working solution by diluting the stock solution 1:8 in 1X PBS. Each sample to be tested requires 5 µL of the Muse Caspase-3/7 working solution.
   a. Dilute Muse Caspase-3/7 stock solution with 1X PBS as suggested in the following table:

   NOTE: Volumes are for one or more extra tests to allow for sufficient volume for the desired number of tests.

Solution	1 Test	5 Tests	10 Tests
Muse® Caspase 3/7 Reagent	1 µL	3.1 µL	6.3 µL
1X PBS	7 µL	21.9 µL	43.7 µL

   b. The Muse Caspase-3/7 Reagent working solution must be used in the same day they are prepared. Store on ice or at 2 to 8°C, protected from light until use.

Muse® Caspase 7-AAD Working Solution

1. Prepare the Muse® Caspase 7-AAD working solution by adding 2 µL of Muse Caspase 7-AAD stock solution to 148 µL of 1X Assay Buffer BA. Each sample to be tested requires 150 µL of the Muse Caspase 7-AAD working solution.
   a. Dilute Muse Caspase 7-AAD stock solution with 1X Assay Buffer BA as suggested in the following table:

NOTE: Volumes are for one or more extra tests to allow for sufficient volume for the desired number of tests.

Solution	1 Test	5 Tests	10 Tests
Muse® Caspase 7-AAD	2 µL	10 µL	20 µL
1X Assay Buffer BA	148 µL	740 µL	1480 µL

b. The Muse Caspase 7-AAD working solution must be used in the same day they are prepared. Store on ice or at 2 to 8°C, protected from light until use.

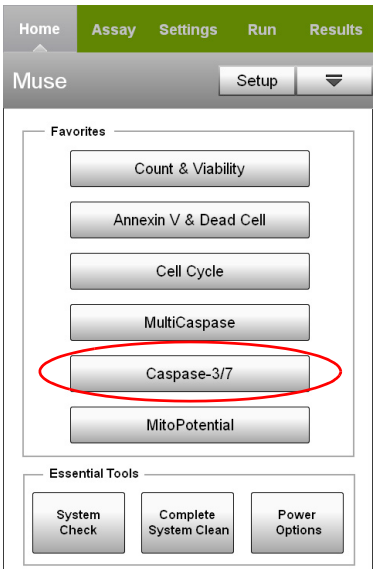
Staining Protocol

1. Allow the Muse® Caspase-3/7 Reagent, 1X Assay Buffer BA, 1X PBS, and Muse Caspase 7-AAD to come to room temperature.
2. Add 50 µL of cells suspension in 1X Assay Buffer BA into each tube. For instructions on preparing cell suspension, see *Appendix A: Cell Sample Preparation* on page 15. Make sure to stain positive and negative controls.
3. Add 5 µL of Muse Caspase-3/7 Reagent working solution to each tube.
4. Mix thoroughly by pipetting up and down or vortexing at a medium speed for 3 to 5 seconds.
5. Loosely cap the tubes and incubate samples for 30 minutes in the 37°C incubator with 5% CO_2.
6. After incubation, add 150 µL of Muse Caspase 7-AAD working solution to each tube.
7. Mix thoroughly by pipetting up and down or vortexing at a medium speed for 3 to 5 seconds.
8. Incubate at room temperature for 5 minutes, protected from light.

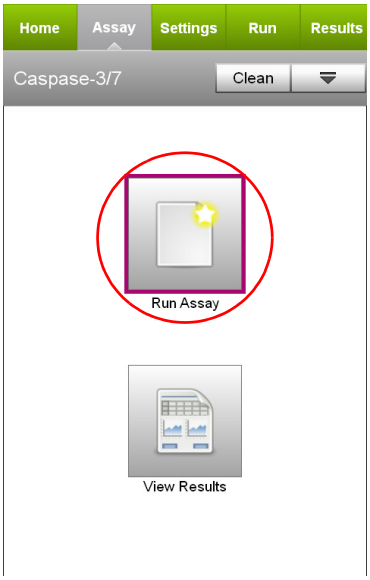
Setup and Acquisition on the Guava® Muse® Cell Analyzer

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*.

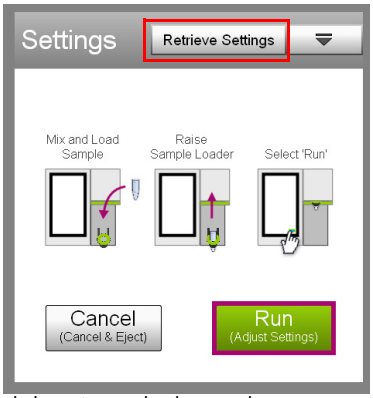
1. Select **Caspase-3/7** from the main menu.



2. Select **Run Assay**.



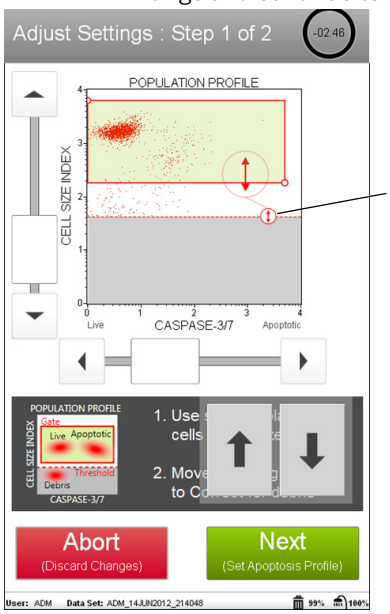
3. Adjust the instrument settings.
   - Load a stained sample for adjusting the settings and select Run. Perform the adjust settings step using a negative control, then verify the settings using a positive control.
   - Or, to retrieve previously saved instrument settings, select Retrieve Settings. For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



4. Fine tune the settings for the CASPASE-3/7 vs CELL SIZE INDEX plot, if necessary.
   - Adjust the CELL SIZE INDEX slider to the left of the plot to move the cell 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.

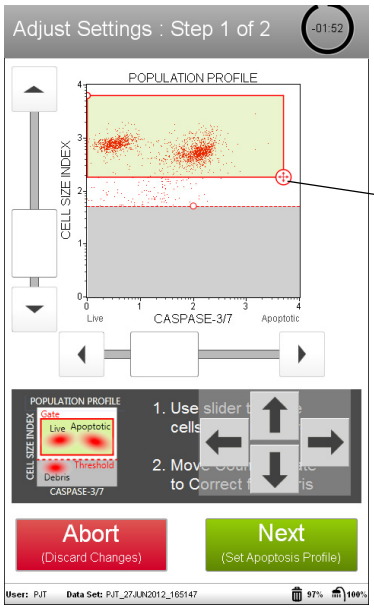
- Drag the upper-left or lower-right corner of the gate to encompass the cell population.

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



Touch threshold to move up/ down.

Negative control sample



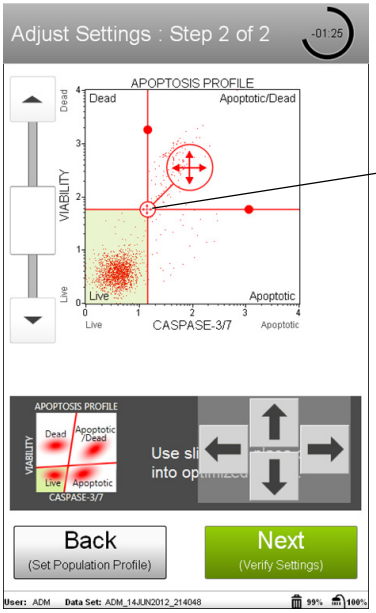
Touch and drag upper-left or lower-right corner to adjust the gate.

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

Positive control sample

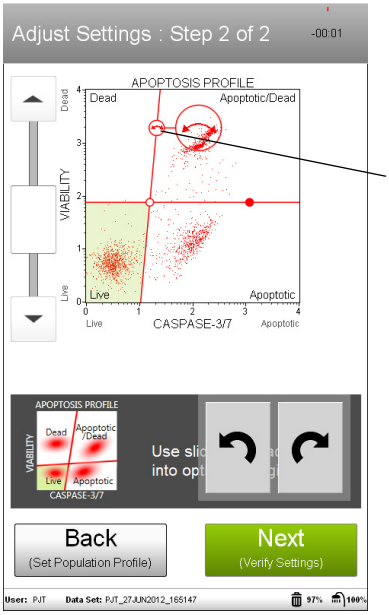
5. Select **Next** when you've completed the adjustments.
6. Fine tune the CASPASE-3/7 vs VIABILITY plot, if necessary.
   - Adjust the Viability slider to place all populations (live, apoptotic and dead) on scale.
   - Adjust the quadrant markers. You can move the marker intersection in any direction, as well as adjust the angle of each line. To move the markers as they are, touch the open circle at the intersection and drag the markers to make large changes, or touch the arrow buttons below the plot to make small changes. To

adjust the angle of either line, touch the solid circle and drag in an arc, or touch the arrow buttons below the plot.



Touch center circle to move the fixed quadrant markers in any direction.

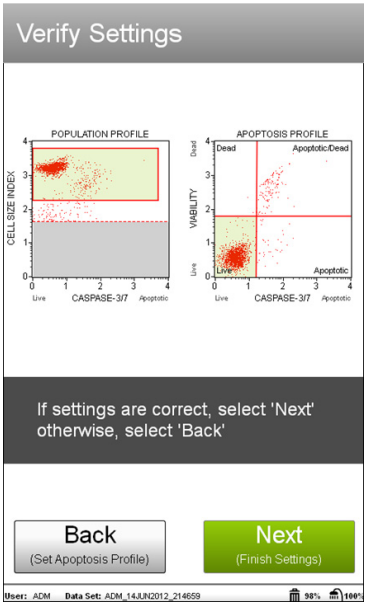
Negative control sample



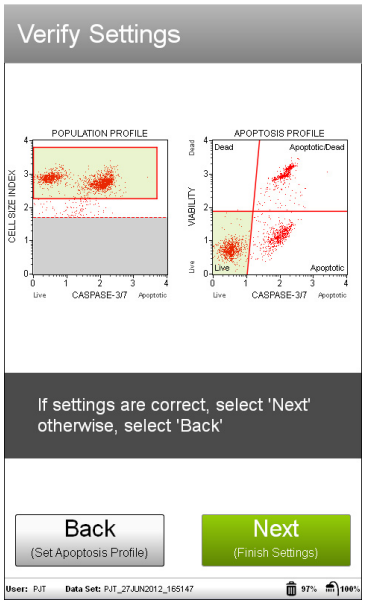
Touch a solid circle on either line (horizontal or vertical) to adjust the angle of the line.

Positive control sample

7. Select **Next** when the adjustments are complete.
8. Verify the settings for the negative control. Then select **Back** and repeat steps 4 through 7 for a positive control. When the settings are correct, select **Next**.

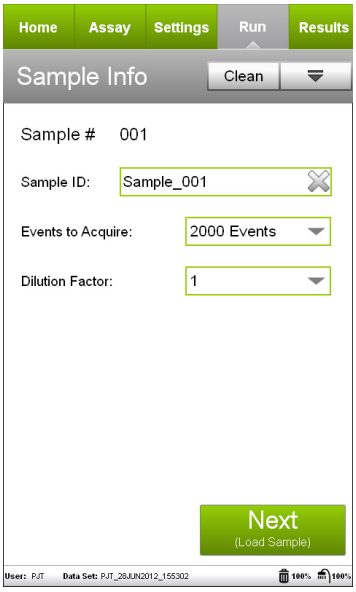


Negative control

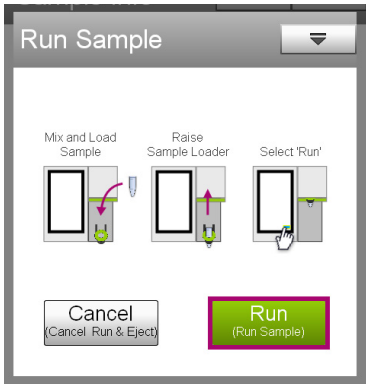


Positive control

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

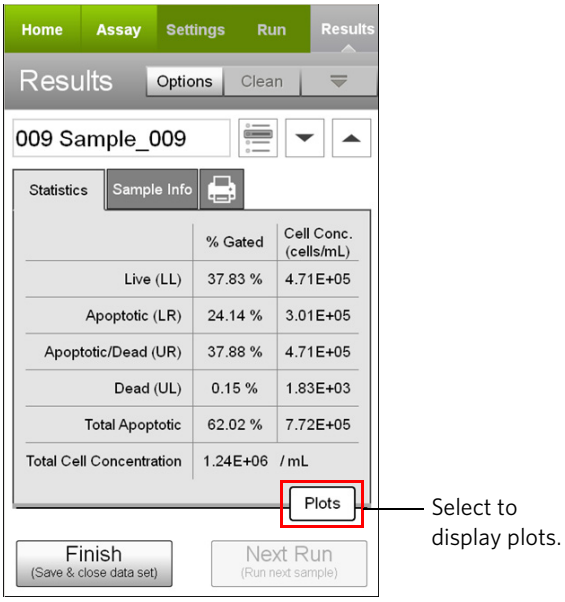


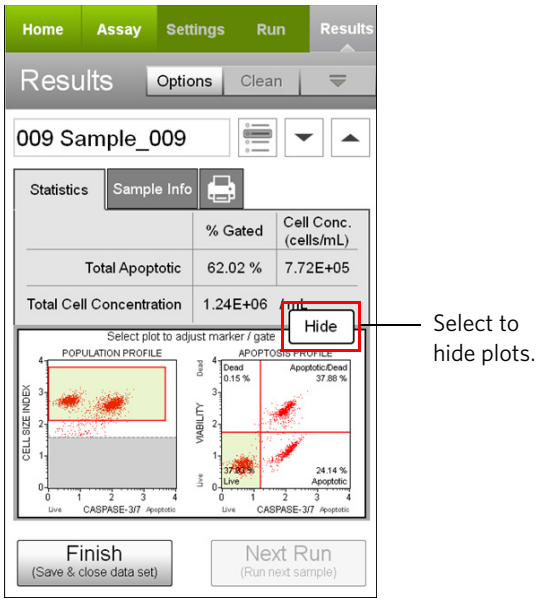
10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.



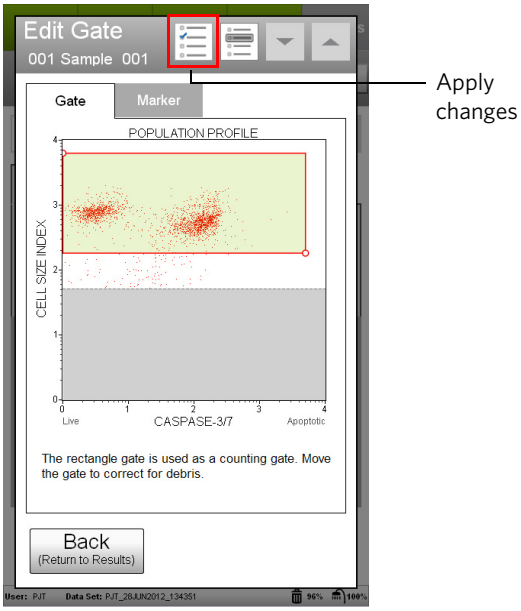
11. When acquisition is complete, the results are displayed. If necessary, select Plots to display dot plots for the sample.

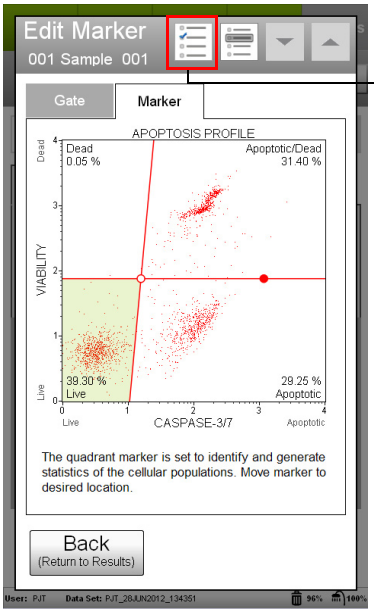
You can view or change the sample ID and dilution factor, as well as add annotations for the current sample by selecting the Sample Info tab. To print the results for the current sample select the printer tab.





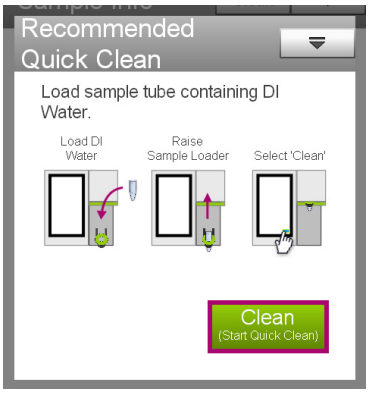
12. (Optional) If changes are needed to the gate or markers, touch a plot to enlarge it, then adjust the cell size gate or markers, as described in steps 4 and 6, respectively. You cannot adjust the cell size threshold after the sample has been acquired.
    If you adjust the gate or markers and wish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

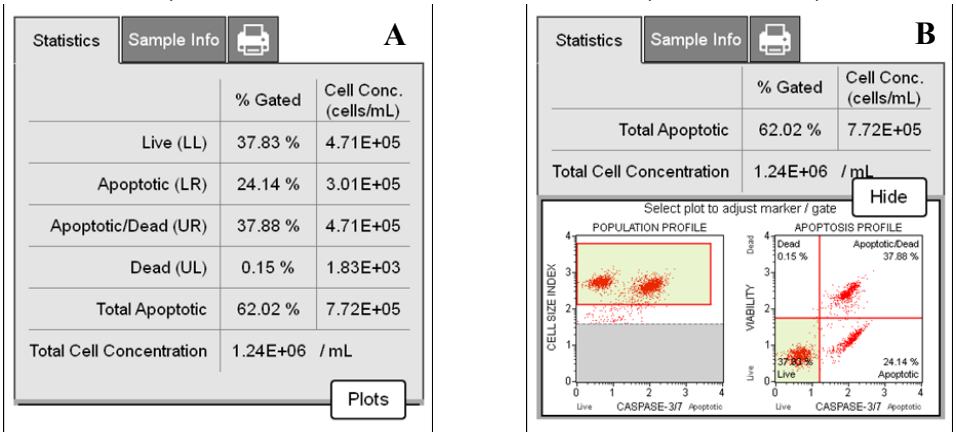
Results

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

- sample number
- sample ID
- percentage of gated cells in each quadrant
    - lower-left: viable cells, not undergoing detectable apoptosis [Caspase-3/7(-) and Dead cell marker (-)]
    - lower-right: cells in the early stages of apoptosis [Caspase-3/7(+) and Dead cell marker (-)]
    - upper-right: cells in the late stages of apoptosis or dead by apoptotic mechanisms [Caspase-3/7(+) and Dead cell marker (+)]
    - upper-left: cells that have died via necrosis but not through the apoptotic pathway [Caspase-3/7(-) and Dead cell marker (+)]
- concentration (cells/mL) of cells in each quadrant
- concentration and percentage of total apoptotic cells (cells in upper-right and lower-right quadrants)
- dilution factor (input value)
- fluorescent intensity values for live and apoptotic cell populations

Figure 2: Jurkat cells were treated with staurosporine to induce apoptosis, then stained with the Muse® Caspase-3/7 Kit and 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 percentages and the concentration (cells/mL) for

the gated events in each quadrant, as well as the percentage and concentration of total apoptotic cells. The first plot in Figure B shows Caspase-3/7 vs Cell Size Index and the second plot shows Caspase-3/7 vs Viability.



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 splashing and cellular breakdown, resulting in volume loss and erroneous results.
2. Multiple acquisitions of a cell sample minimize sampling error. Statistically, multiple acquisitions yield more accurate cell count and viability results.
3. The default number of events to acquire is 2000. You may select a different number; however, your statistical error will increase as you decrease the number of events for acquisition.
4. If the cell count results deviate from expected values, check that the correct value was entered for the dilution factor. The Caspase-3/7 application can be used to recalculate cell counts. Open the data file corresponding to the mistaken entry. Reenter the correct dilution factor and the cell count values will be recalculated automatically.
5. 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.
6. 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, add additional buffer to bring the volume up to 100 µL 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 a 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.
7. The Caspase-3/7 assay 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 Caspase-3/7 assay with a "clumpy" cell type, 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.luminexcorp.com for detailed information on the Muse Cell Dispersal Reagent and assay method. For more troubleshooting tips, refer to the *Guava Muse Cell Analyzer User's Guide*.

IMPORTANT: This assay requires that your Guava Muse Cell Analyzer has software version 1.3 or later installed for accurate statistical analysis. Check the software version on your Muse Instrument and update, if necessary. The latest version of Muse Software can be downloaded free of charge from www.luminexcorp.com.

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. The cell concentration is too high. Decrease the number of cells/µL by diluting the sample to 300–500 cells/µL. The Guava® Muse® Cell Analyzer gives the most accurate data when the flow rate is less than 500 cells/µL.
Instrument clogging; too many cells	Run a Quick Clean to clean out the 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. Ensure that cells are counted properly prior to beginning the experiment.
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. Dilute the sample further with 1X Assay Buffer BA to adjust the cell concentration below 500 cells/µL. For best results, we recommend a cell concentration of 200–300 cells/µL.
Background staining and/or non-specific staining of cells	If all samples appear to be induced even when low levels of induction are expected, your cultured may be compromised. It is important to run negative control samples for each experiment. The negative control should be a sample from your cell culture, not treated to induce apoptosis. Typically, negative control samples show a low level of Caspase-3/7 and/or dead cell marker–positive cells that are distinct from that of induced cells, because healthy cell cultures contain a small number of apoptotic and/or dead cells. However, sub-optimal culture conditions may stress cells in culture, causing them to undergo apoptosis in the absence of experimental induction treatment. The negative control from a stressed culture often shows increased Caspase-3/7 and/or dead cell marker reactivity.

Potential Problem	Experimental Suggestion
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. • If there are low levels of Caspase-3/7 staining, the Muse Caspase-3/7 Reagent may be degraded. Verify proper storage and handling of the Muse® Caspase-3/7 Reagents. Prepare fresh Caspase-3/7 Reagent working solution just before staining cells. • If there are no Caspase-3/7 positive cells, it is possible that your cells may not be fully induced or the Caspase-3/7 may not have been taken up correctly by the cells. The Caspase-3/7 staining results can vary over time as apoptosis progresses. To determine optimal apoptotic induction, conduct a time-course study to achieve the best results for Caspase-3/7 staining. Also, positive control samples are recommended for each experiment. Positive controls should be appropriate for comparison with the test procedure or test cell population. Use a cell line previously characterized as inducible for apoptosis. Treatments used to induce apoptosis in various cell lines include serum starvation; activation of cell surface receptors such as Fas, TNFR1, or TCR; UV irradiation; and treatment with a compound known to induce apoptosis in your cell line.
Poor separation between live and apoptotic populations	• If the separation between populations is poor, the concentration of Caspase-3/7 Reagent may be too low. Muse Caspase-3/7 Reagent has been formulated for optimal performance using Jurkat, CHO, HeLa, and PC3 cells. Other cells may show different patterns of reactivity that require adjustments to the amount of reagent used. For best results, titer the Muse Caspase-3/7 Reagent to determine the amount for maximal staining of cells. • If the separation between populations is dimmer than expected, it is possible that the stained samples or the Caspase-3/7 Reagent may have been exposed to light. Repeat the staining using fresh reagents. Avoid prolonged exposure of reagents and stained samples to light.
Percent of apoptotic cells increases over time	If the percent of apoptotic and/or dead cells increases significantly over time, it is possible that samples are not stable over the time required to acquire the data. The Caspase-3/7 Assay uses live cells, hence, the staining profiles are dynamic and can change as the cell sample ages. Stability of the stained cells can vary among cell lines. Extended storage of stained samples may adversely affect results. We recommend acquiring samples within 2 hours after preparation.
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 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 to verify proper instrument function and accuracy.

Limitations

- The results of the assay are dependent upon proper handling of samples, reagents, and instruments.
- Cell types vary in the caspase content. The amount of caspase expressed intracellularly varies among cell types after apoptosis is induced.
- The Muse® Caspase-3/7 Kit is designed for use on unfixed cells. Fixing cells may yield inaccurate results.
- The Guava® Muse Cell Analyzer and Muse Caspase-3/7 Kit yield optimal results when the stained cell sample used for acquisition is between 2 x 10^4 to 5 x 10^5 cells/mL. To obtain the most accurate results, adjust the cell concentration to within the recommended range. However, to optimize throughout, Luminex recommends using between 2 x 10^5 to 1 x 10^6 cells/mL when possible.

Appendix A: Cell Sample Preparation

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, treatment, and the removal of the inducing medium, cells are resuspended at a concentration of 1 x 10^5 to 5 x 10^6 cells/mL in the 1X Assay Buffer BA provided.

   NOTE: The process of removing the inducing medium and resuspending cells in 1X Assay Buffer BA may cause cellular loss and differences in concentrations from the original culture.

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, 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. Resuspend the cells in 1X Assay Buffer BA so the final concentration is between 1 x 10^5 to 5 x 10^6 cells/mL.
3. Proceed to *Staining Protocol* on page 5.

References

1. Riedl SJ, Shi Y. Molecular mechanisms of caspase regulation during apoptosis. *Nat Rev Mol Cell Biol*. 2004;5:897-907.
2. Cohen GM. Caspases: the executioners of apoptosis. *Biochem J*. 1997;326:1–16.
3. Portera AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*. 1999;6:99-104.

4. Benetti L, et al. NucView™ 488 Caspase-3 Substrate for Live cells - Biotium Product Insert. *J Virology*. 2007:10242-10248.
5. Leuenroth SJ, Okuhara D, Shotwell JD, Markowitz GS, Yu Z, Somlo S, Crews CM. Triptolide is a traditional Chinese medicine-derived inhibitor of polycystic kidney disease. *Proc Natl Acad Sci USA*. 2007;104:4389-4394.
6. Tribulatti MV, Mucci J, Cattaneo V, Aguero F, Gilmartin T, Head SR, Campetella O. Galectin-8 induces apoptosis in the CD4 (high) CD8 (high) thymocyte subpopulation. *Glycobiology*. 2007;17:1404-1412.

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
- Muse® Cell Dispersal Reagent – MCH100107
- Muse® MultiCaspase Kit – MCH100109
- Muse® MitoPotential Kit – MCH100110

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