Muse™ MultiCaspase Kit
User's Guide

Catalog No. MCH100109 (100 Tests)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

IMPORTANT: This assay requires that your 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.millipore.com/muse.

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Application

The Muse™ MultiCaspase Kit allows for the facile, rapid, and quantitative measurements of two important cell health parameters simultaneously—caspase activation and cellular plasma membrane permeabilization and cell death. The assay provides relative percentages of cells that are live, exhibiting caspase activity, and dead, for both adherent and suspension cell lines on the 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, caspase(+), caspase(+) and dead, total caspase(+), and dead cells.
- Cell Concentrations (cells/mL) for live, caspase(+), caspase(+) and dead, and dead cells

The Muse™ MultiCaspase 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. In addition several caspases have also been found to have non-apoptotic roles in nonapoptotic functions of caspases in inflammation, mediating immunity, cell fate specification, cell survival, cell cycle regulation, cell proliferation, and cell migration.[2] 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).[3]

Muse™ MultiCaspase Assay is a Pan Caspase assay that can detect the presence of multiple caspases (caspase-1, 3, 4, 5, 6, 7, 8, and 9).[4] The assay simultaneously determines the count and percentage of cells with caspase activity, in combination with a dead cell dye. The kit utilizes a derivatized VAD-peptide[5] that can detect the activity of multiple caspases, and a dead cell dye that provides information on membrane integrity or cell death.

- The VAD peptide is derivatized with a fluorescent group and a FMK moiety and called Fluorescent-Labeled Inhibitor of Caspases (FLICA).[6] The peptide is membrane permeable and non-cytotoxic. The peptide binds to activated caspases with resulting fluorescent signal proportional to the number of active caspases in the cell that appears as increased signal in the Caspase axis.

- 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 but permeates later stage apoptotic and dead cells. Dead cells thus show increased fluorescence on the Viability axis.

Four populations of cells can be distinguished:

- (LL) Live cells: Caspase(-) and 7-AAD(-)
- (LR) Caspase(+) cells exhibiting Pan Caspase activity: Caspase(+) and 7-AAD(-)
- (UR) Late stage of Caspase activity cells: Caspase(+) and 7-AAD(+)
- (UL) Necrotic cells: Caspase(-) and 7-AAD(+)

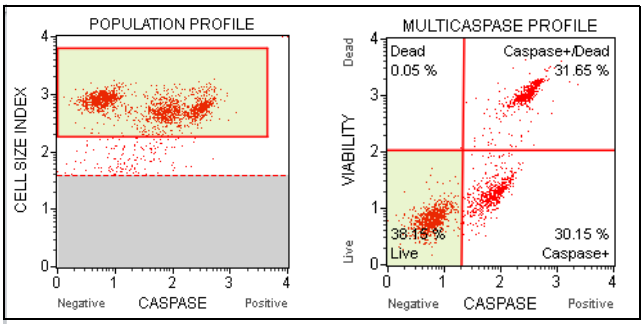
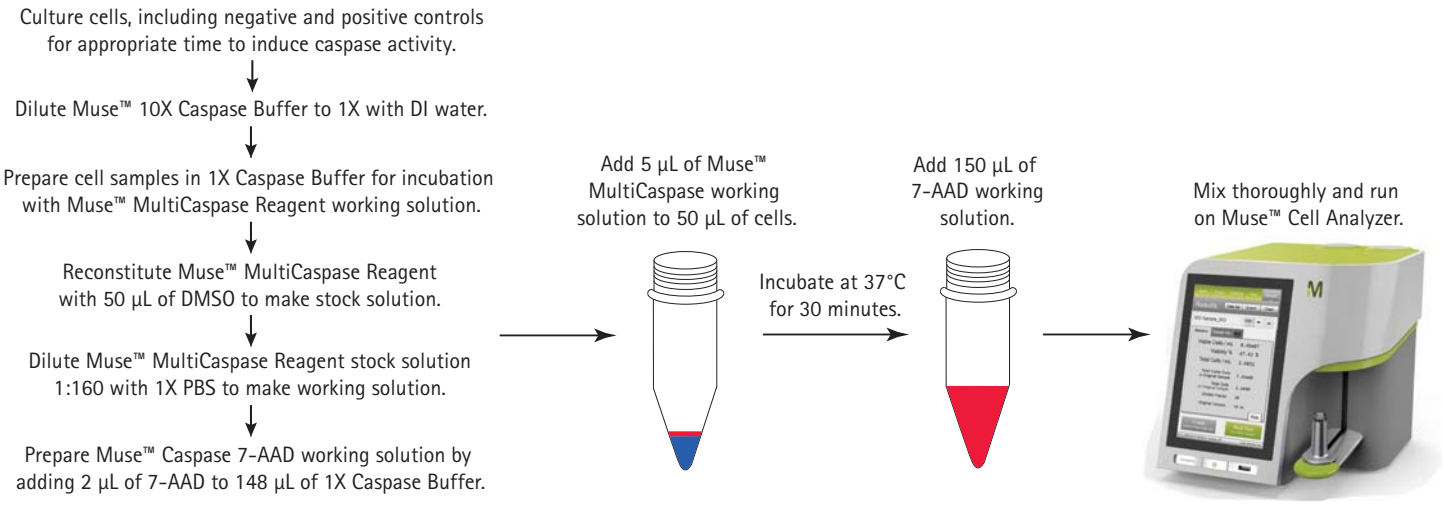


Figure 1. Representative plots from the Muse™ MultiCaspase Assay. The first plot has a threshold marker, allowing you to eliminate debris based on cell size, as well as a gate to include cells. The second plot shows the gated cells with quadrant markers providing data on four cell populations—Live, Caspase(+), Caspase(+)/Dead, and Dead cells.

Summary of Protocol



Kit Components

- Muse™ MultiCaspase Reagent (Part No. 4700-1530, 100 tests/bottle)
- Muse™ Caspase 7-AAD (Part No. 4700-1510, 100 tests/bottle)
- Muse™ 10X Caspase Buffer (Part No. 4700-1535, 100 tests/bottle)
- 1X PBS (Part No. 4700-1515, 100 tests/bottle)
- Anhydrous DMSO (Part No. 4300-0160, 100 tests/bottle)

Materials Required but Not Supplied

- Muse™ Cell Analyzer
- Cell suspension treated and untreated to induce caspase activity
- 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™ MultiCaspase Kit is intended for research use only.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
- The Muse™ MultiCaspase 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 MSDS for specific information on hazardous materials.
- The 10X Caspase Buffer and 1X PBS contain 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 of the solution.
- 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. Stored 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™ MultiCaspase 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™ MultiCaspase 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 2 hours after staining with the Muse™ MultiCaspase Reagent, the stability of individual cell types may vary.

Time considerations: The process of staining cells with the Muse™ MultiCaspase Reagent and the recommended protocol takes 30 to 45 minutes. Acquiring data on your Muse™ Cell Analyzer takes less than 3 minutes per sample, depending on the cell concentration and desired number of events to acquire. However, preparing cells for testing

may require periodic maintenance and cultivation. Once you cultivate the proper number of cells for the experiment, it takes an additional 2 to 48 hours of culture with various inducers to stimulate detectable caspase activity.

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 13.

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

Reagent Preparation

Muse™ 1X Caspase Buffer

The Muse™ Caspase Buffer is supplied as a 10X concentrate, which must be diluted to 1X with DI water prior to use. Approximately 300 µL of 1X Muse™ Caspase Buffer is required per sample to be stained.

1. Warm the Muse™ 10X Caspase Buffer to room temperature to completely dissolve any crystals that may have formed during storage.
2. Mix 1 part of Muse™ 10X Caspase Buffer with 9 parts of DI water.
3. Gently vortex to mix.

Muse™ MultiCaspase Reagent Stock Solution

The Muse™ MultiCaspase Reagent is supplied lyophilized. It must be reconstituted with DMSO to make stock solution.

1. Add 50 µL DMSO to the Muse™ MultiCaspase Reagent vial.
2. There is a small amount of powder in each vial, so thoroughly mix the vial by repeatedly inverting until the reagent is completely dissolved. This may take a few minutes.

   NOTE: To recover all reconstituted reagent, we recommend centrifuging the vial for 1 minute at 200 x g.
3. Immediately use the volume of stock solutions necessary to prepare the required amount of working solution (see below)
4. Aliquot the remaining Muse™ MultiCaspase Reagent stock solution and store at –15 to –25°C, desiccated and protected from light.

   NOTE: Avoid more than two freeze/thaw cycles of the reconstituted Muse™ MultiCaspase Reagent stock solution since the solvent will take up moisture and cause deterioration of the reagent.

Muse™ MultiCaspase Reagent Working Solution

Prepare the Muse™ MultiCaspase Reagent working solution by diluting the stock solution 1:160 in 1X PBS. Each sample to be tested requires 5 µL of the MultiCaspase working solution.

1. Dilute Muse™ MultiCaspase Reagent 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.

Component	1 to 10 Tests	10 to 40 Test	40 to 60 Tests	60 to 100 Tests
Muse™ MultiCaspase Reagent stock solution	1 µL	2 µL	3 µL	4 µL
1X PBS	159 µL	318 µL	477 µL	636 µL

2. The MultiCaspase Reagent working solution must be used the same day it is 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 Caspase Buffer. Each sample to be tested requires 150 µL of the Muse™ Caspase 7-AAD working solution.
2. Dilute Muse™ Caspase 7-AAD stock solution with 1X Caspase Buffer as suggested in the following table:

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

Component	1 Test	5 Tests	10 Tests
Muse™ Caspase 7-AAD	2 µL	10 µL	20 µL
1X Caspase Buffer	148 µL	740 µL	1480 µL

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

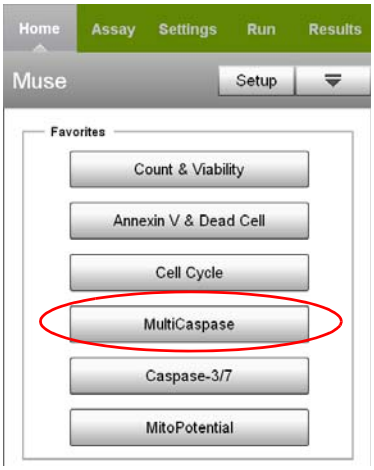
Staining Protocol

1. Add 50 µL of cells in suspension to each tube. For instructions on preparing cell suspension, see "Appendix A: Cell Sample Preparation" on page 13. Make sure to stain positive and negative controls.
2. Add 5 µL of Muse™ MultiCaspase Reagent working solution to each tube.
3. Mix thoroughly by pipetting up and down or vortexing at a medium speed for 3 to 5 seconds.
4. Loosely cap the tubes and incubate samples for 30 minutes in the 37°C incubator with 5% CO_2.
5. After incubation, add 150 µL of Muse™ Caspase 7-AAD working solution to each tube.
6. Mix thoroughly by pipetting up and down or vortexing at a medium speed for 3 to 5 seconds.
7. Incubate at room temperature for 5 minutes, protected from light.

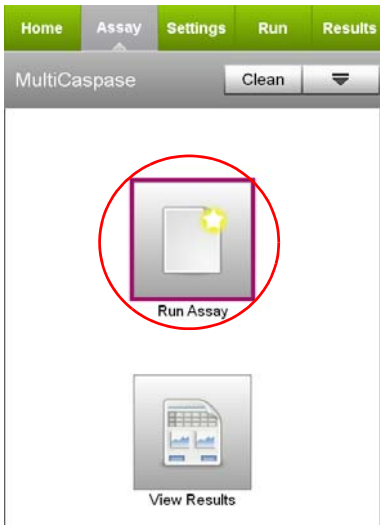
Setup and Acquisition on the Muse™ Cell Analyzer

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

1. Select **MultiCaspase** from the main menu.



2. Select **Run Assay**.

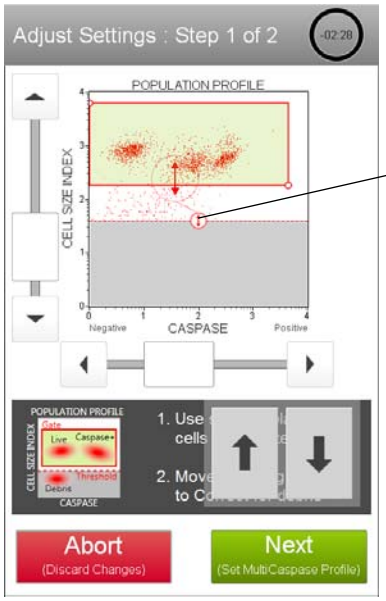


3. Adjust the instrument settings.

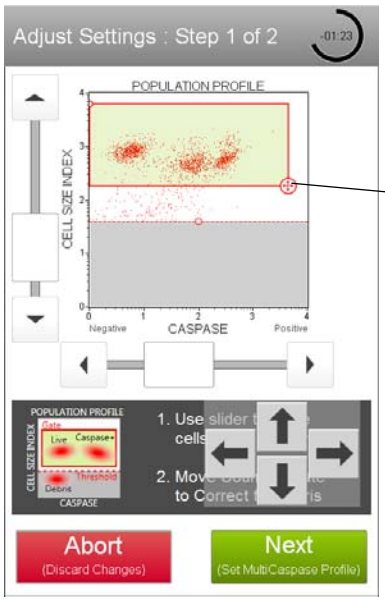
   - Load a stained sample for adjusting the settings and select **Run**.
   - Or, to retrieve previously saved instrument settings, select **Retrieve Settings**. For more information on retrieving settings, see the *Muse™ Cell Analyzer User's Guide*.

4. Fine tune the settings for the CASPASE 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), remove the tube and mix well before reloading and continuing. Then, select either **Back** to restart the adjust settings step, or **Next** to accept the settings and continue to the next step.



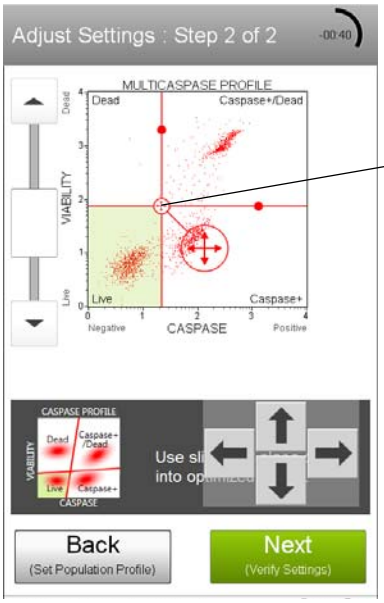
Touch threshold to move up/down.



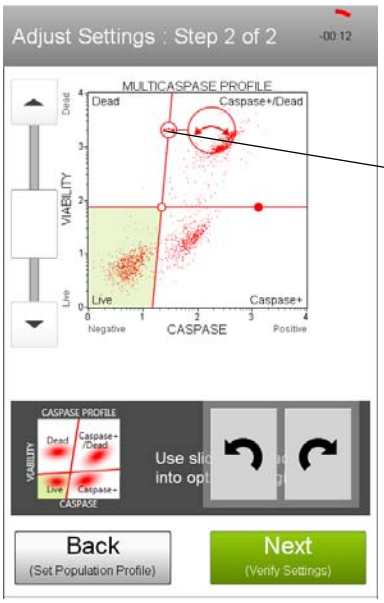
Touch and drag upper-left or lower-right corner to adjust the gate.

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

5. Select **Next** when you've completed the adjustments.
6. Fine tune the settings for the CASPASE vs VIABILITY plot, if necessary.
   - Adjust the Viability slider to place all populations (live, caspase[+], 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.

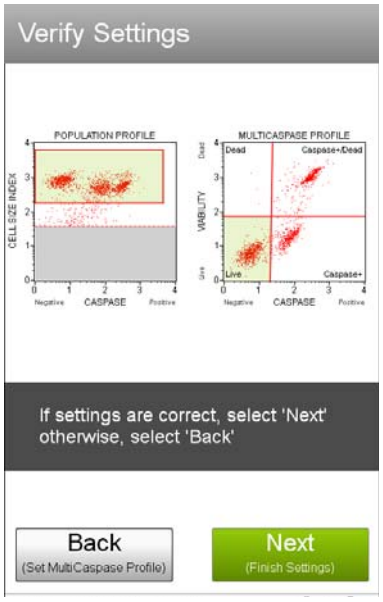


Touch center circle to move the fixed quadrant markers in any direction.



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

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.

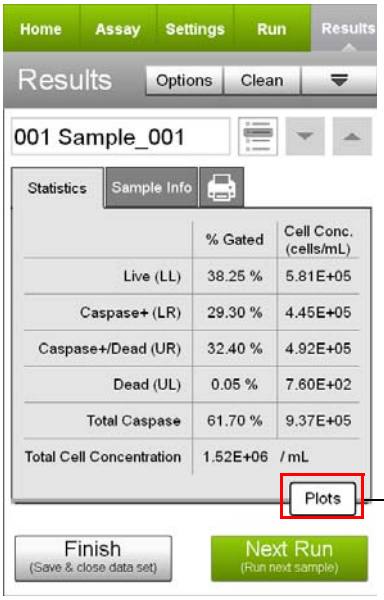


9. Enter the sample ID by touching the field, then using the keypad to input the ID. Touch **Done** when you finish entering the ID. If necessary, change the **Events to Acquire** and/or **Dilution Factor** by touching the field, then selecting the value from the pop-up menu. Select **Next**.

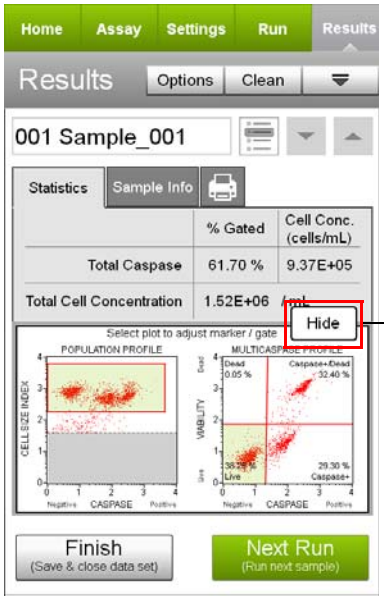
10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.

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/or 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.



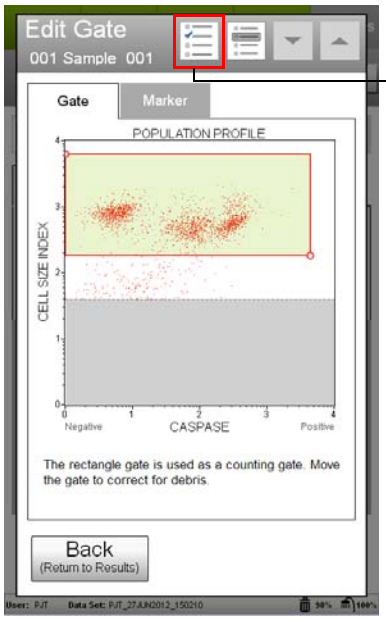
Select to display plots.



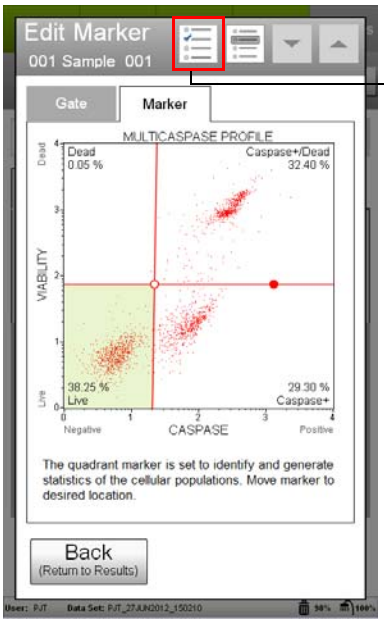
Select to hide plots.

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

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



Apply changes



Select to apply changes to other samples.

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

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

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

Results

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:

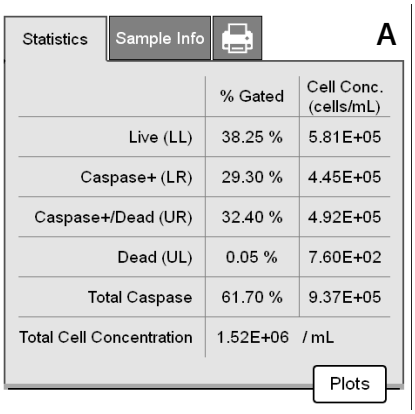
- sample number
- sample ID
- percentage of gated cells in each quadrant

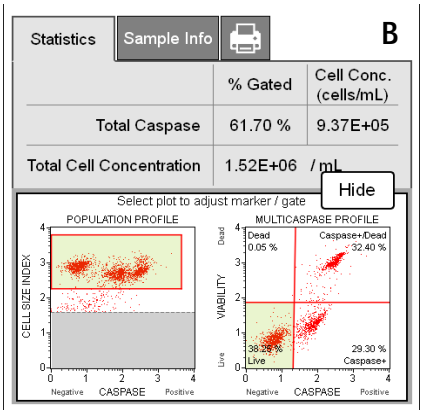
  lower-left: viable cells, not exhibiting caspase activity [Caspase (-) and Dead cell marker (-)]

  lower-right: cells exhibiting caspase activity [Caspase (+) and Dead cell marker (-)]

  upper-right: cells in the late stages of caspase activity or dead by caspase mechanisms [Caspase (+) and Dead cell marker (+)]

  upper-left: cells that have died via necrosis but not through the caspase pathway [Caspase (-) and Dead cell marker (+)]
- concentration (cells/mL) of cells in each quadrant
- concentration and percentage of total caspase-positive cells (cells in upper-right and lower-right quadrants)
- dilution factor (input value)
- fluorescent intensity values for live and caspase-positive cell populations





Figures A and B. Jurkat cells were treated with staurosporine, then stained with the Muse™ MultiCaspase Kit and acquired on the Muse™ Cell Analyzer. Figure A shows summary data, while Figure B shows results displayed with optional dot plots. The statistics show the percentages and the concentration (cells/mL) for the gated events in each quadrant, as well as the percentage and concentration of total caspase-positive cells. The first plot in Figure B shows Caspase vs Cell Size Index and the second plot shows Caspase 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 MultiCaspase 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 *Muse™ Cell Analyzer User's Guide* for additional troubleshooting tips, or contact Millipore Technical Support for help.
7. The MultiCaspase 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™ MultiCaspase 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.millipore.com/muse for detailed information on the Muse™ Cell Dispersal Reagent and assay method. For more troubleshooting tips, refer to the *Muse™ Cell Analyzer User's Guide*.
8. IMPORTANT: This assay requires that your 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.millipore.com/muse.

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

Troubleshooting

Potential Problem	Experimental Suggestions
Acquisition taking longer than expected or progress bar stops during acquisition	Ensure that the System Check procedure was run and passed. If the progress bar stops during acquisition, the fluid system may be clogged. Run a Quick Clean procedure. The cell concentration is too high. Decrease the number of cells/µL by diluting the sample to 300–500 cells/µL. The 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 Caspase Buffer 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 caspase activity. Typically, negative control samples show a low level of caspase and/or dead cell marker–positive cells that are distinct from that of induced cells, because healthy cell cultures contain a small number of activated caspase and/or dead cells. However, sub-optimal culture conditions may stress cells in culture, causing them to undergo caspase activation in the absence of experimental induction treatment. The negative control from a stressed culture often shows increased caspase and/or dead cell marker reactivity.
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 staining, the Muse™ MultiCaspase Reagent may be degraded. Verify proper storage and handling of the Muse™ MultiCaspase Reagents. Prepare fresh MultiCaspase Reagent working solution just before staining cells. • If there are no caspase-positive cells, it is possible that your cells may not be fully induced or the MultiCaspase may not have been taken up correctly by the cells. The MultiCaspase staining results can vary over time as apoptosis progresses. To determine optimal caspase activation, conduct a time-course study to achieve the best results for MultiCaspase 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 caspase activation. Treatments used to induce caspase activation 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 caspase activation in your cell line.

Potential Problem	Experimental Suggestions
Poor separation between live and caspase(+) populations	• If the separation between populations is poor, the concentration of MultiCaspase Reagent may be too low. The 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™ MultiCaspase 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 MultiCaspase Reagent may have been exposed to light. Repeat the staining using fresh reagents. Avoid prolonged exposure of reagents and stained samples to light.
Percentage of caspase(+) cells increases over time	If the percent of caspase(+) 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 MultiCaspase 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 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 caspase activation is induced.
- The Muse™ MultiCaspase Kit is designed for use on unfixed cells. Fixing cells may yield inaccurate results.
- The Muse™ Cell Analyzer and Muse™ MultiCaspase 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, EMD Millipore 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 1x 10^5 to 5 x 10^6 cells/mL in 1X Caspase Buffer (see page 4 for preparation instructions).

   NOTE: The process of removing the inducing medium and resuspending cells in 1X Caspase Buffer 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 Caspase Buffer 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.

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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™ Caspase-3/7 Kit – MCH100108
- Muse™ MitoPotential Kit – MCH100110

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