



# Muse™ Human CD8 T Cell Kit

## User's Guide

*Catalog No. MIM100102 (100 Tests)*

**CAUTION:** This kit should not be used for patient diagnosis or patient management.

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures.



---

## Application

The Muse™ Human CD8 T Cell Kit is used in laboratory research studies to determine the CD8 T-cell count, total lymphocyte count, and CD8 T-cell percent of lymphocytes in human whole blood and peripheral mononuclear cell (PBMC) samples. Identification and enumeration of cytotoxic CD8 T-cell populations are useful in understanding immune response and status, and in the research of disease mechanisms, such as autoimmune diseases, immunodeficiency. Minimal sample preparation is required in this no-wash assay to obtain accurate and precise results. The software provides:

- CD8 T-cell concentration in cells/ $\mu$ L
- CD8 T-cell percent of lymphocytes
- Total lymphocyte concentration in cells/ $\mu$ L

The Muse™ Human CD8 T Cell Assay is for use with the Muse™ Cell Analyzer. The Muse™ System makes sophisticated fluorescent-based analysis fast, easy, convenient, and affordable. Sample preparation is minimal, and after loading samples onto the Muse™ Cell Analyzer, the 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.

**CAUTION:** This kit should not be used for patient diagnosis or patient management.

For Research Use Only; not for use in diagnostic procedures.

---

## Test Principle

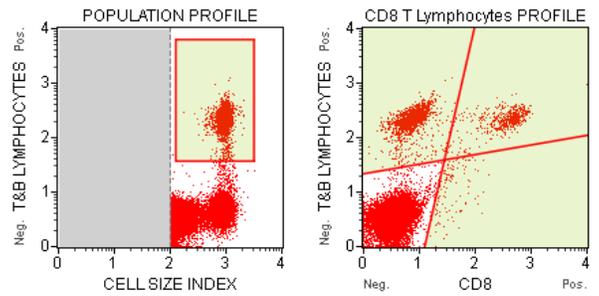
CD8 T lymphocytes (cytotoxic or killer T cells) are an important population of T lymphocytes<sup>1</sup> that play a critical role in host defense by their cytolytic capability to destroy cells that are damaged or infected with virus or bacteria, or tumor cells. They act by inducing programmed apoptosis in target cells.<sup>2</sup>

Normal CD8 T cells make up between 10% and 36% of the lymphocytes in healthy adults and show alteration of percent levels with age and development.<sup>3-4</sup> CD8 T cells contribute to the eradication of intracellular infection and contribute to the control of many chronic infections. Identification and enumeration of cytotoxic CD8 T-cell populations are useful in research studies to understand the mechanisms of autoimmune diseases,<sup>5-6</sup> chronic pulmonary diseases,<sup>7</sup> hepatitis B infections,<sup>8</sup> as well as in research of immune reaction in transplant rejection or vaccine studies.<sup>9</sup>

The Muse™ Human CD8 T Cell Kit allows for the detection and identification of lymphocytes and CD8 T lymphocytes in either whole blood or PBMCs using a simplified no-wash assay. The CD8 antibody allows the identification of CD8, a 68-kDa transmembrane glycoprotein expressed by class I major histocompatibility complex restricted, mature suppressor/cytotoxic T cells, the great majority of cortical thymocytes and approximately 30% of medullary thymocytes. In addition a proportion of  $\gamma\delta$  T cells and NK cells express CD8. Inclusion of several proprietary antibodies in the anti-lymphocyte cocktail allows for the unique identification of the CD8 cytotoxic T cells.

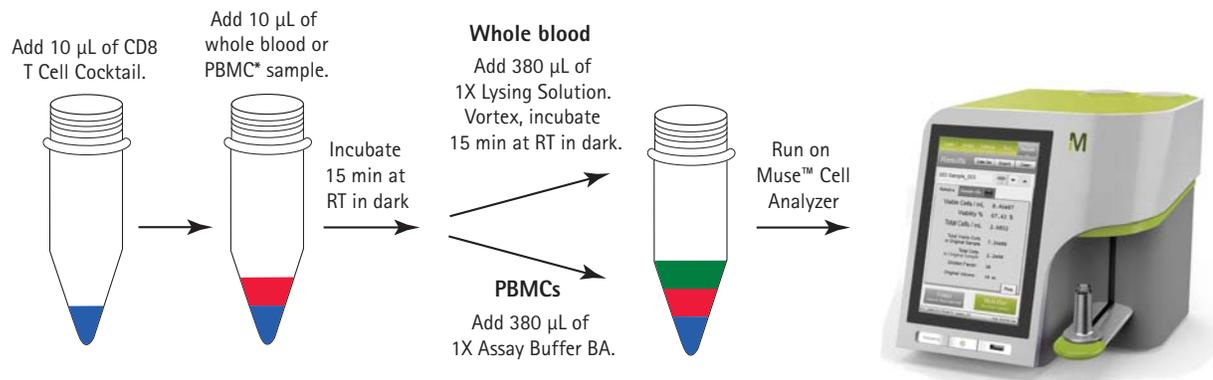
The Muse™ CD8 T Cell Assay uses an anti-lymphocyte cocktail that identifies the lymphocyte population, and CD8 antibody that binds to the CD8 cells. The assay provides results for CD8 T lymphocytes, CD8 percent of lymphocytes, and total lymphocyte counts. Results in each of the four quadrants are indicated.

- UL: CD8(-) T&B lymphocytes [T&B Lymphocyte(+) and CD8(-)]
- UR: CD8(+) T Lymphocytes [T&B Lymphocyte(+) and CD8(+)]
- LL: Negative Cells [T&B Lymphocyte(-) and CD8(-)]
- LR: CD8(+) Cells [T&B Lymphocyte(-) and CD8(+)]



**Figure 1.** Plots from the Muse™ CD8 T Cell Assay. The first plot has a threshold marker, allowing you to eliminate debris based on cell size, as well as a gate to include lymphocytes. The second plot shows quadrant markers providing data on CD8 T lymphocytes.

## Summary of Protocol



\* PBMC samples should be in 1X Assay Buffer BA at a concentration of  $4 \times 10^5$  to  $2 \times 10^7$  cells/mL.

## Kit Components

- Muse™ Human CD8 T Cell Cocktail (Part No. 4700-1625, 100 tests/vial)
- Human 1X Lysing Solution (Part No. 4700-1620, 100 tests/vial)
- 1X Assay Buffer BA (Part No. 4700-1360, 100 tests/vial)

## Materials Required but Not Supplied

- Muse™ Cell Analyzer
- Whole blood or PBMC samples
- Micropipettors
- Disposable micropipettor tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog No. 16466-030, or equivalent)
- Vortex mixer
- Disposable gloves
- 100% bleach solution
- Deionized water
- Muse™ System Check Kit (Catalog No. MCH100101)

- Muse™ Count & Viability Reagent (Catalog No. MCH100102)
  - Guava Fixative (Catalog No. 4700-0140), optional
  - Guava ICF Instrument Cleaning Fluid (Catalog No. 4200-0140), optional
- 

## Precautions

- The Muse™ Human CD8 T Cell Kit is intended for research use only; not for use in diagnostic procedures. This kit should not be used for patient diagnosis or patient management.
  - Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this product.
  - The 1X Assay Buffer BA and Muse™ CD8 T Cell Cocktail 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 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.
  - Some assay components included in the kit may be harmful. Please refer to the MSDS sheet for specific information on hazardous materials.
  - All fluorochrome conjugated antibodies are light-sensitive and must be stored in the dark at 2 to 8°C.
  - During storage and shipment, small volumes of product may become entrapped in the seal of the product vial. For maximum recovery of product, centrifuge vial briefly prior to removing the cap.
  - Avoid microbial contamination of the reagents, 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.
  - Do not use the reagent beyond the expiration date.
  - Material Safety Data Sheets (MSDS) for kit reagents are available from our website ([www.millipore.com/muse](http://www.millipore.com/muse)), by contacting Millipore Technical Support, or from the Millipore technical library at [www.millipore.com/techlibrary](http://www.millipore.com/techlibrary).
- 

## Storage

- Store the Human CD8 T Cell Cocktail and 1X Assay Buffer BA at 2 to 8°C. Protect the CD8 T Cell Cocktail from exposure to light.
- Store the Guava 1X Lysing Solution at room temperature (18 to 25°C).

**CAUTION:** Fluorochrome-conjugated antibodies should always be stored at 2 to 8°C. Any deviation in temperature for long periods of time may compromise the performance of the antibodies.

---

## Before You Begin

Acquire samples shortly after the sample preparation is complete. While some donors have been shown to yield stable results for up to 4 hours, the stability of individual donors may vary. This time variability is a consequence of using live, unfixed cells. You should determine the stability results for your own samples. If longer stability is necessary, Guava Fixative may be added to the 1X Human Lysing Solution or the 1X Assay Buffer BA, as outlined in "Staining Protocol" below.

This protocol was developed to allow direct enumeration of CD8 T cells in whole blood and PBMCs in cultures. Optimal staining is obtained for cell concentrations between  $4 \times 10^5$  to  $2 \times 10^7$  cells/mL. Millipore recommends using the Muse™ Count & Viability Reagent to obtain accurate cell counts. Care should be taken to keep cell concentrations as constant as possible in all samples of an experiment.

**Time considerations:** The process of staining cells with the Muse™ Human CD8 T Cell Kit takes approximately 30 minutes for whole blood samples and 15 minutes for PBMCs. Acquiring data on the Muse™ Cell Analyzer takes approximately 2 minutes per sample.

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

---

## Specimen Collection

Collect blood by venipuncture into a sterile K<sub>3</sub> EDTA (lavender top) or sodium heparin (green top) blood collection tube.

**CAUTION:** Discard blood samples that are hemolyzed, clotted, lipemic, discolored, or containing interfering substances.

Stain blood within 30 hours of collection for optimal results. Unstained anticoagulated blood should be maintained at 18 to 25°C prior to sample processing.

Leave the capped tube of blood standing upright or lying on its sides if it is stored overnight. Do not rock or agitate blood in any way during extended storage.

---

## Staining Protocol

Batch your preparations to avoid over-incubation of samples. Unfixed samples must be acquired within 4 hours after preparation. Samples fixed with 0.2% Guava fixative, capped, and stored refrigerated are stable for 24 hours.

1. Pipette 10 µL of Human CD8 T Cell Cocktail into each tube.

**CAUTION:** Place the stock bottle of Human CD8 T Cell Cocktail in the refrigerator or on ice immediately after use. Do not allow the bottle of cocktail to remain at elevated temperatures for an extended time.

2. Add 10 µL of sample to each tube.

**For whole blood samples:** Blood in the collection tubes should be thoroughly resuspended by gentle agitation for a few minutes before removing an aliquot for sample preparation.

**For PBMC samples:** Optimal staining is obtained for cell concentrations between  $4 \times 10^5$  to  $2 \times 10^7$  cells/mL. Resuspend cells in 1X Assay Buffer BA at appropriate concentrations.

3. Mix the samples thoroughly by pipetting up and down.

**CAUTION:** Avoid leaving blood to dry on the side of the tubes. This may cause erroneous results.

4. Incubate the samples for 15 minutes at room temperature (18 to 25°C) in the dark.

5. After incubation, add the following:

**For whole blood samples:** Add 380  $\mu\text{L}$  of Human 1X Lysing Solution directly to each tube to bring the final volume to 400  $\mu\text{L}$ .

**For PBMC samples:** Add 380  $\mu\text{L}$  of room temperature 1X Assay Buffer BA directly to each tube to bring the final volume to 400  $\mu\text{L}$ .

**(Optional Protocol with Guava Fixative):** If fixation is desired, add 4  $\mu\text{L}$  of Guava Fixative per 400  $\mu\text{L}$  of 1X Lysing Solution or 1X Assay Buffer to a final concentration of 0.2% and use as above.

6. Immediately mix the samples thoroughly by pipetting up and down.

7. **For whole blood samples:** Incubate for a minimum of 15 minutes at room temperature (18 to 25°C) in the dark. After incubation, samples are ready to run on the Muse™ Cell Analyzer.

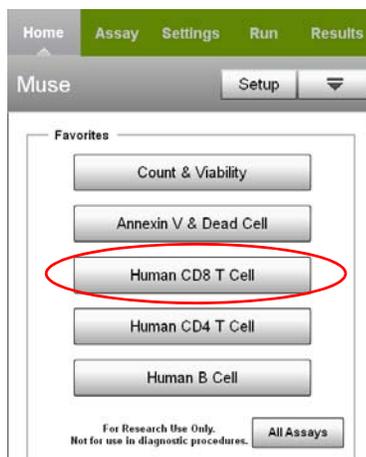
**For PBMC samples:** Run on the Muse™ Cell Analyzer.

---

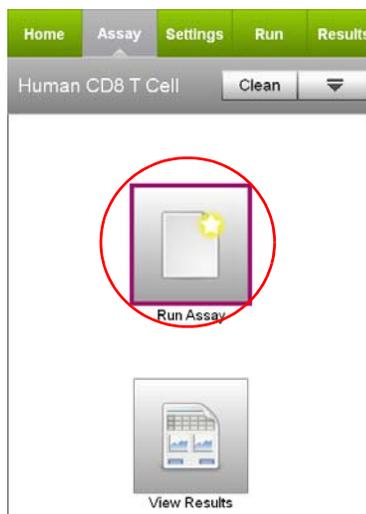
## Setup and Acquisition on the Muse™ Cell Analyzer

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 **Human CD8 T Cell** from the main menu.

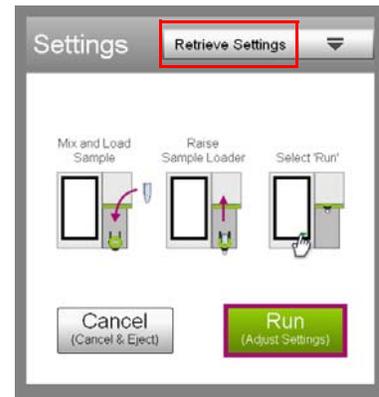


2. Select **Run Assay**.



3. Adjust the instrument settings.

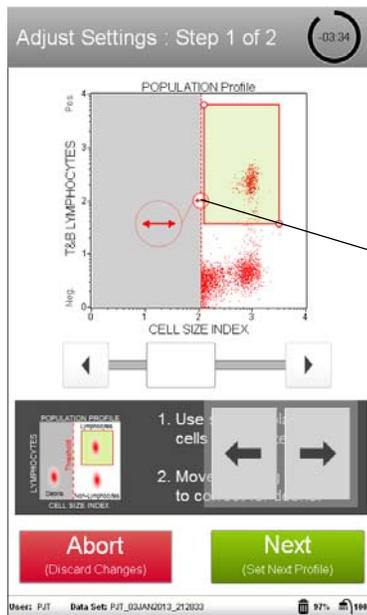
- Load a stained sample for adjusting the settings and select **Run**. Perform the adjust settings steps using a stained sample.
- Or, to retrieve previously saved instrument settings, select **Retrieve Settings**. For more information on retrieving settings, see the *Muse™ Cell Analyzer User's Guide*.



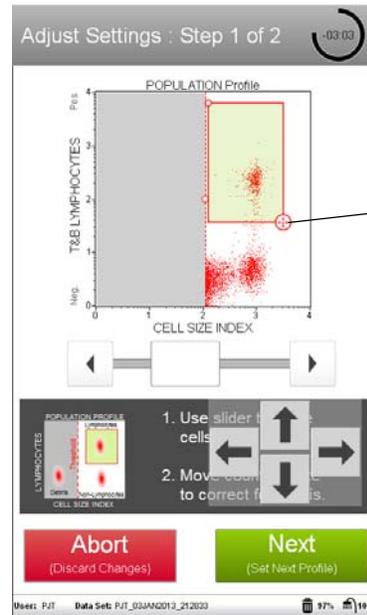
4. Fine tune the settings for the CELL SIZE INDEX vs LYMPHOCYTE plot, if necessary.

- Adjust the CELL SIZE INDEX slider below the plot to move the lymphocyte 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 adjust settings step 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 right/left.



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

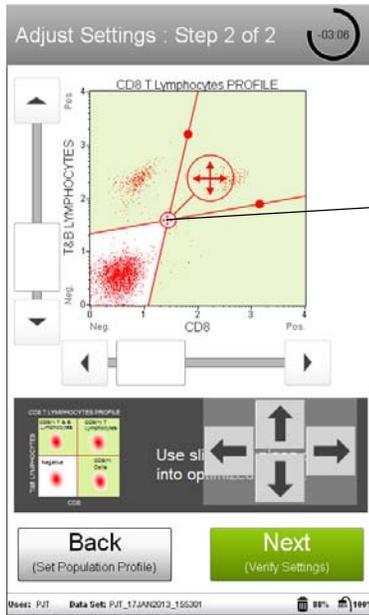
This example data shows typical gate and marker settings. The threshold was raised to remove debris.

5. Select **Next** when you've completed the adjustments.

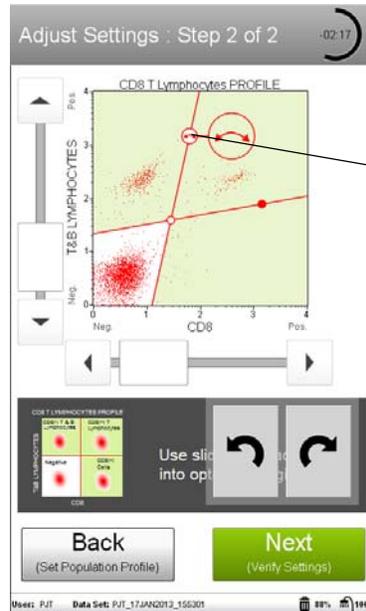
6. Fine tune the settings for the CD8 vs LYMPHOCYTES plot, if necessary.

- Adjust the x and y-axis sliders to position the CD8(-) and CD8(+) lymphocytes in the upper-left and upper-right quadrants (green area).
- Adjust the quadrant markers, if necessary. 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.

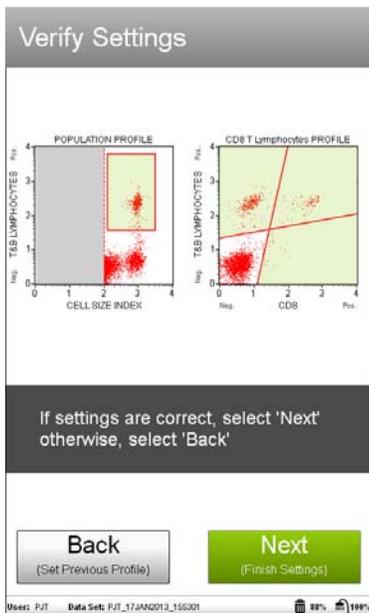


Touch center to move the fixed quadrant markers in any direction.

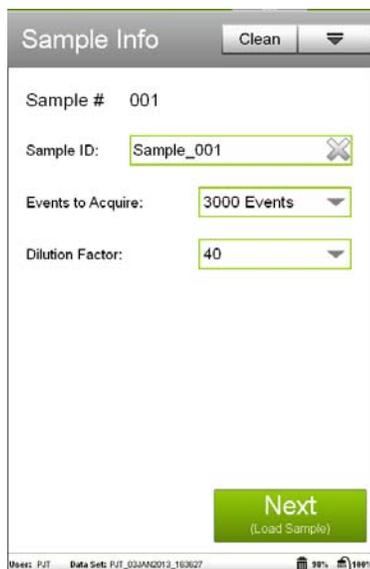


Touch a solid circle on either line (horizontal or vertical) to adjust the angle of the line.

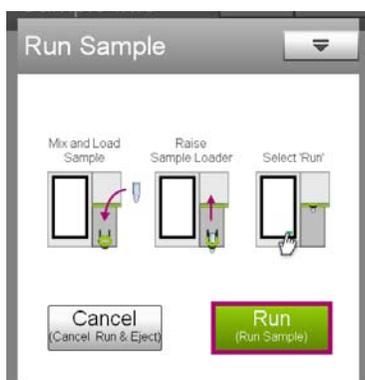
7. Select **Next** when the adjustments are complete.
8. Verify the settings for the control sample. Select **Back** to make further adjustments. When the settings are correct, select **Next**.



9. Enter the sample ID for the first sample 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**.

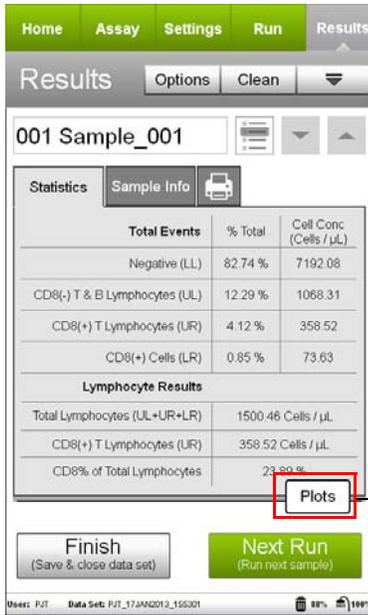


10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.

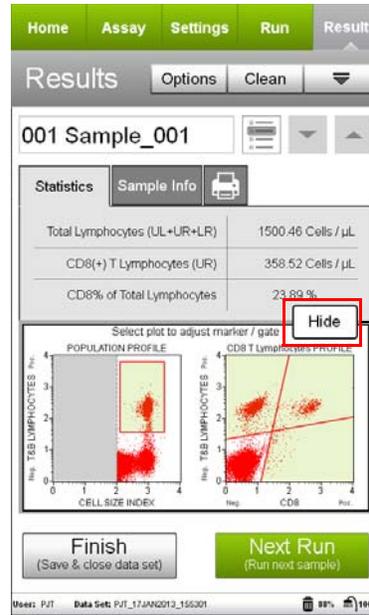


11. When acquisition is complete, the results are displayed. Select **Plots** to display dot plots for the sample.

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.



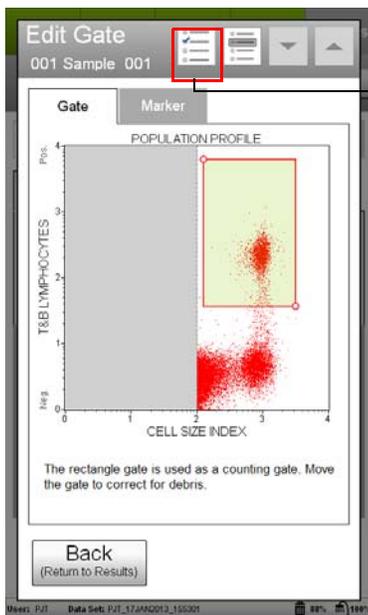
Select to display plots.



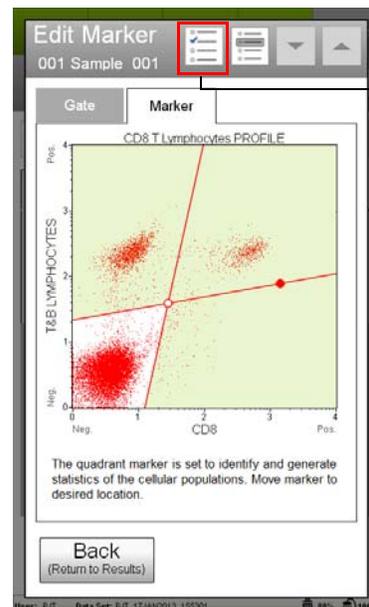
Select to hide plots.

12. (Optional) If changes are needed to the gate or markers, touch a plot to enlarge it, then adjust the lymphocyte 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.



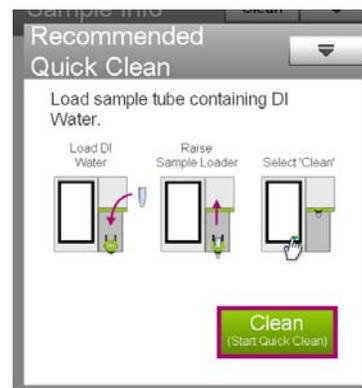
Apply changes



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.

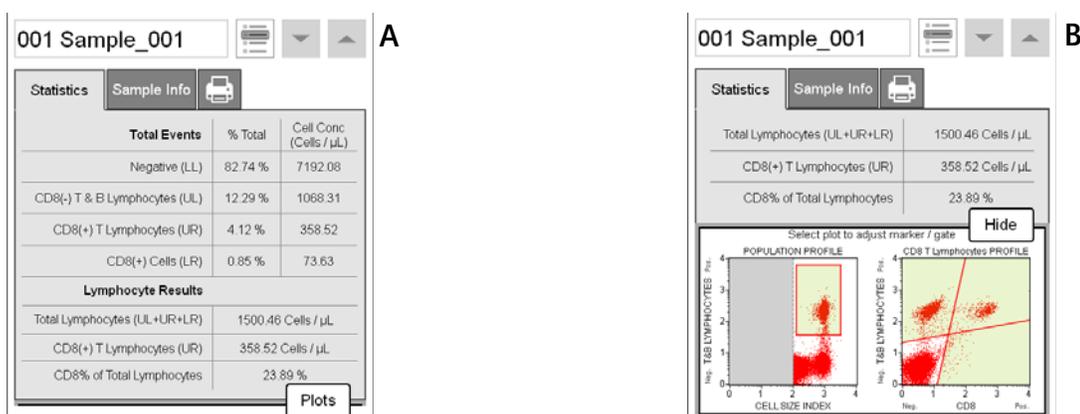


14. When you have acquired the last sample, select **Finish**, enter a name for the data set or leave the default and select **OK**.
15. (Optional) Select **Options** in the title bar to rename the data set, export the data set, save the current instrument settings, or view the event log. Refer to the *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:

- sample number
- sample ID
- percentage of cells in each quadrant
  - upper-left:** CD8(-) T and B lymphocytes [T&B lymphocyte(+) and CD8(-)]
  - upper-right:** CD8(+) T lymphocytes [T&B lymphocyte(+) and CD8(+)]
  - lower-left:** Negative cells [T&B lymphocyte(-) and CD8(-)]
  - lower-right:** CD8(+) cells [T&B lymphocyte(-) and CD8(+)]
- concentration and percentage of cells in each quadrant
- CD8 T-lymphocyte concentration, total lymphocyte concentration, and CD8 T-cell percent of lymphocytes
- dilution factor (input value)



**Figures A and B.** Whole blood was stained with the Muse™ Human CD8 T Cell 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 concentrations (cells/ $\mu$ L) for the results in each quadrant out of the total events. The Lymphocyte Results show the total lymphocyte concentration, the CD8 T-lymphocyte concentration, and the CD8 cell percentage of lymphocytes. The first plot in Figure B shows Cell Size Index vs Lymphocytes and a lymphocyte gate, and the second plot shows CD8 vs Lymphocytes.

---

## Technical Tips

1. All kit reagents, Human CD8 T Cell Cocktail, 1X Assay Buffer BA, and 1X Human Lysing Solution should be brought to room temperature prior to staining.
2. For cellular staining and analysis to be most effective, make sure that test cells have good viability prior to use.
3. To clean the instrument after running whole blood, perform a Complete System Clean using two tubes of water instead of Guava ICF and water. Follow with a second Complete System Clean cycle using Guava ICF, then water, as directed on the screen. See *Muse System User's Guide* for details.

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](http://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](http://www.millipore.com/techservice).

---

## Troubleshooting

Potential Problem	Experimental Suggestions
Acquisition rate decreases dramatically; instrument clogging; too many cells	<ul style="list-style-type: none"><li>• Cell concentration too high: For PBMCs, decrease the cells per microliter by diluting sample to 300-500 cells/uL. The Muse™ system gives the most accurate data when the flow rate is less 500 cells/uL. For whole blood, increase the dilution factor using the Human 1X Lysing Solution to 80.</li><li>• 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.</li></ul>
Too few cells	<ul style="list-style-type: none"><li>• Restain sample using 180 µL of Human 1X Lysing Solution. Ensure dilution factor in the software is set to 20.</li><li>• If using PBMCs, ensure that enough cells were stained as described in "Staining Protocol" on page 4. Restain sample using a more concentrated stock cell suspension.</li></ul>
Background staining and/or non-specific staining of cells	<ul style="list-style-type: none"><li>• Ensure sample was mixed thoroughly prior to acquiring.</li><li>• Ensure dried blood from side of tube was removed prior to staining. Unstained blood will contribute to erroneous results.</li></ul>
Low level of staining	<ul style="list-style-type: none"><li>• Ensure sample was stained for a minimum of 15 minutes followed by complete lysis for 15 minutes.</li><li>• Treated donors may show less separation of cellular populations. Ensure a control sample is run.</li></ul>
Variability in day-to-day and sample-to-sample experiments	<ul style="list-style-type: none"><li>• 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.</li><li>• Ensure that samples are lysed for at least 15 minutes with room temperature Human 1X Lysing Solution.</li><li>• If there appears to be day-to-day variation of the staining pattern, ensure the Muse™ Cell Analyzer is working properly. Run the System Check procedure using the System Check Kit (Part No. MCH100101) to verify proper instrument function and accuracy.</li></ul>

---

## References

1. Zhang N, Bevan MJ. CD8(+) T cells: foot soldiers of the immune system. *Immunity*. 2011;35:161-168.
2. Berke G. The binding and lysis of target cells by cytotoxic lymphocytes: molecular and cellular aspects. *Annu Rev Immunol*. 1994;12:735-773.
3. Denny T, Yogev R, Gelman R, Skuza C, et al. Lymphocyte subsets in healthy children during the first 5 years of life. *JAMA*. 1992;267:1484-1488.
4. Hulstaert F, Hannet I, Deneys V, Munhyeshuli V. Age-related changes in human blood lymphocyte subpopulations: II. Varying kinetics of percentage and absolute count measurements. *Clin Immunol Immunopathol*. 1994;70:152-158.
5. Asano M, Toda M, Sakaguchi N, Sakaguchi S. Autoimmune disease as a consequence of developmental abnormality of a T cell subpopulation. *J Exp Med*. 1996;184:387-396.
6. Blanco P, Viallard JF, Pellegrin JL, Moreau JF. Cytotoxic T lymphocytes and autoimmunity. *Curr Opin Rheumatol*. 2005;17:731-734.
7. Kemeny D, Vyas B, Vukmanovic-Stejic M, Thomas M, et al. T cell subsets and chronic obstructive pulmonary disease. *J Respir Crit Care Med*. 1999;160:S33-S37.
8. Thimme R, Wieland S, Steiger C, Ghayeb J, et al. CD8(+) T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. *J Virol*. 2003;77:68.
9. Appay V, Douek DC, Price DA. CD8+ T cell efficacy in vaccination and disease. *Nat Med*. 2008;14:623-628.

---

## Related Kits

- Muse™ Human CD4 T Cell Kit - MIM100101
- Muse™ Human B Cell Kit - MIM100103
- Muse™ Human Lymphocyte CD69 Kit - MIM100104
- Muse™ Human Lymphocyte CD25 Kit - MIM100105
- Muse™ System Check Kit - MCH100101
- Muse™ Count & Viability Kit (100T) - MCH100102
- Muse™ Annexin V & Dead Cell Kit - MCH100105

---

## Warranty

**EMD Millipore Corporation** ("Millipore") warrants its products will meet their applicable published specifications when used in accordance with their applicable instructions for either: a) a period of one year from the date of shipment of the products; or b) a term less than one year, as such term is expressly stated on the product specification or product label. **MILLIPORE MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.** The warranty provided herein and the data, specifications, and descriptions of Millipore products appearing in Millipore's published catalogues and product literature may not be altered except by express written agreement signed by an officer of Millipore. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized, and if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Millipore's sole obligation shall be to repair or replace, at its option, the applicable product or part thereof, provided the customer notifies Millipore promptly of any such breach. If after exercising reasonable efforts, Millipore is unable to repair or replace the product or part, then Millipore shall refund to the Company all monies paid for such applicable Product. **MILLIPORE SHALL NOT BE LIABLE FOR CONSEQUENTIAL, INCIDENTAL, SPECIAL, OR ANY OTHER DAMAGES RESULTING FROM ECONOMIC LOSS OR PROPERTY DAMAGE SUSTAINED BY ANY COMPANY CUSTOMER FROM THE USE OF ITS PRODUCTS.**

© 2013 EMD Millipore Corporation. All rights reserved. No part of these works may be reproduced in any form without permission in writing.

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption or application to humans or animals.