Muse™ Human Lymphocyte CD25 User's Guide

Catalog No. MIM100105 (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.

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Application

The Muse™ Human Lymphocyte CD25 Kit is for use in laboratory research studies to determine the CD25 lymphocyte count, total lymphocyte count, and CD25 percent of total lymphocytes in human whole blood and PBMC samples. CD25 (IL-2 receptor α-chain) is an activation marker expressed on the surface of activated lymphocytes including T and B lymphocytes and NK cells. It is also expressed constitutively on the surface of small sub-populations of T cells. The study of CD25 expression on lymphocytes is important in understanding immune response and development, in immuno-modulatory mechanisms, and in the research of disease mechanisms.

- CD25(+) lymphocyte concentration in cells/µL
- CD25 percent of total lymphocytes
- Total lymphocyte concentration in cells/µL

The Muse™ Human Lymphocyte CD25 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.

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Test Principle

Activation of lymphocytes is a complex and regulated sequence of events that leads to the expression of cytokine receptors, the secretion of cytokines, and the expression of specific cell surface molecules that leads to divergent immune responses.[1-3] The study of lymphocyte activation with stimuli such as antibodies, cytokines, and polyclonal mitogens has become critical in understanding immuno-modulatory mechanisms and immune response, and in research to understand the mechanisms of diseases.

Multiple proteins are known to be upregulated during the immune activation process. The more common of these proteins include early activation marker CD69, CD71 (early), CD25 (mid), and mid to late Class II HLA-DR. CD25 (Interleukin-2 receptor α chain) is a 55-kDa type I transmembrane glycoprotein expressed on activated B, T, and NK lymphocytes,[4-6] activated monocytes/macrophages, and constitutively on a sub-population of CD4 T lymphocytes (T regulatory cells). CD25 elevation on lymphocyte populations has been observed under a number of conditions where antigenic stimulation was used. Antigen density increases on phytohemagglutinin (PHA)-, concanavalin A (Con A)-, and CD3-activated T lymphocytes; multiple studies have looked at the kinetics of CD69 and CD25 expression and cytokine secretion.[6-8] Changes in CD25 expression levels have also been studied in the mechanism of different diseases such as chronic heart disease, psoriasis vulgaris, and autoimmune disease.[9-12]

The Muse™ Human Lymphocyte CD25 Kit detects and identifies total lymphocytes and CD25 lymphocytes in either whole blood or PBMCs using a simplified no-wash assay.

The Muse™ Human Lymphocyte CD25 Assay uses an anti-lymphocyte cocktail that identifies the total lymphocyte population and a CD25 antibody that identifies mid to late activation marker CD25. The kit also includes an isotype control antibody. The assay provides results for CD25(+) lymphocytes, CD25 percentage of total lymphocytes, and the total lymphocyte count. Results in each of the four quadrants are indicated.

- UL: Non-CD25 Lymphocytes [Lymphocyte(+) and CD25(-)]
- UR: CD25(+) Lymphocytes [Lymphocyte(+) and CD25(+)]
- LL: Negative Cells [Lymphocyte(-) and CD25(-)]
- LR: CD25(+) Non-Lymphocytes [Lymphocyte(-) and CD25(+)]

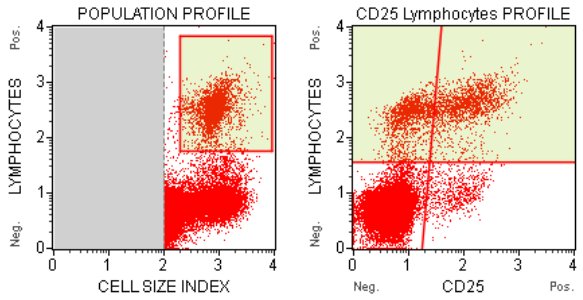
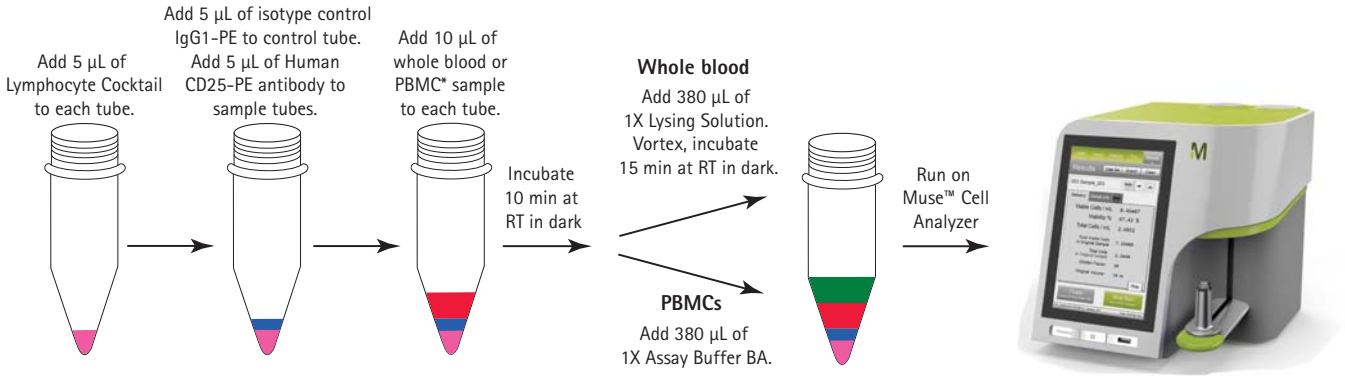


Figure 1. Representative plots from the Muse™ Human Lymphocyte CD25 Assay. The first plot has a threshold marker, allowing you to eliminate debris based on cell size, as well as a gate to include lymphocytes. The second plot shows quadrant marker providing data on CD25 lymphocytes.

Summary of Protocol



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

Kit Components

- Muse™ Human Lymphocyte Cocktail (Part No. 4700-1650, 100 tests/vial)
- Muse™ Human CD25-PE Antibody (Part No. 4700-1660, 100 tests/vial)
- Isotype Control Mouse IgG1-PE (Part No. 4700-1390, 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 Lymphocyte CD25 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.
- All kit components, except the Human 1X Lysing Solution, 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 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 reagents beyond the expiration date.
- Material Safety Data Sheets (MSDS) for kit reagents are available from our website (www.millipore.com/muse), by contacting Millipore Technical Support, or from the Millipore technical library at www.millipore.com/techlibrary.

Storage

Store the Guava 1X Lysing Solution at room temperature (18 to 25°C).

Store the following kit components at 2 to 8°C:

- Muse™ Human Lymphocyte Cocktail
- Muse™ Human CD25-PE Antibody
- Isotype Control Mouse IgG1-PE
- 1X Assay Buffer BA

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 CD25 cells in whole blood and PBMCs in cultures. Optimal staining is obtained for cell concentrations between 4×10^5 to 2×10^7 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 Lymphocyte CD25 Kit takes approximately 25 minutes for whole blood samples and 10 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_3 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 5 µL of Muse™ Human Lymphocyte Cocktail into each tube.

   CAUTION: Put the stock bottle of Human Lymphocyte Cocktail back into 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. Pipette 5 µL of Isotype Control Mouse IgG1-PE into each control tube.

3. Pipette 5 µL of Muse™ Human CD25-PE Antibody into each sample tube.

   CAUTION: Put the stock bottles of Isotype Control Mouse IgG1-PE and Human CD25-PE Antibody back into the refrigerator or on ice immediately after use. Do not allow the antibody and control to remain at elevated temperatures for an extended time.

4. 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 4×10^5 to 2×10^7 cells/mL. Resuspend cells in 1X Assay Buffer BA at appropriate concentrations.

5. 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.

6. Incubate the samples for 10 minutes at room temperature (18 to 25°C) in the dark.

7. After incubation, add the following:

   For whole blood samples: Add 380 µL of Human 1X Lysing Solution directly to each tube to bring the final volume to 400 µL.

   For PBMC samples: Add 380 µL of room temperature 1X Assay Buffer BA directly to each tube to bring the final volume to 400 µL.

   NOTE: (Optional Protocol with Guava Fixative): If fixation is desired, add 4 µL of Guava Fixative per 400 µL of 1X Lysing Solution or 1X Assay Buffer to a final concentration of 0.2% and use as above.

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

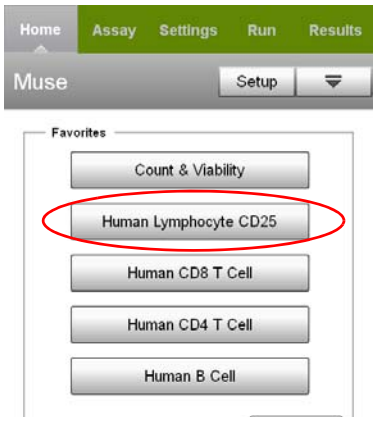
9. **For whole blood samples:** Incubate for a minimum of 15 minutes at room temperature (18 to 25°C) in the dark. After incubation, samples are ready to run on the Muse™ Cell Analyzer.

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

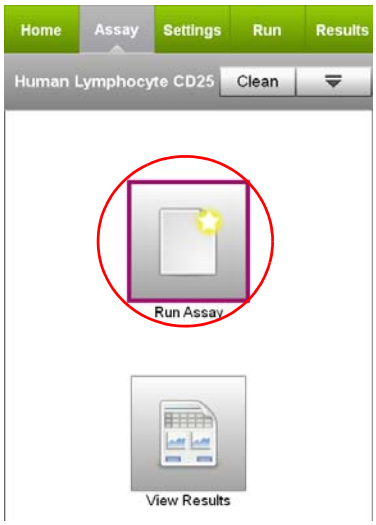
Setup and Acquisition on the Muse™ Cell Analyzer

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 Lymphocyte CD25** from the main menu.



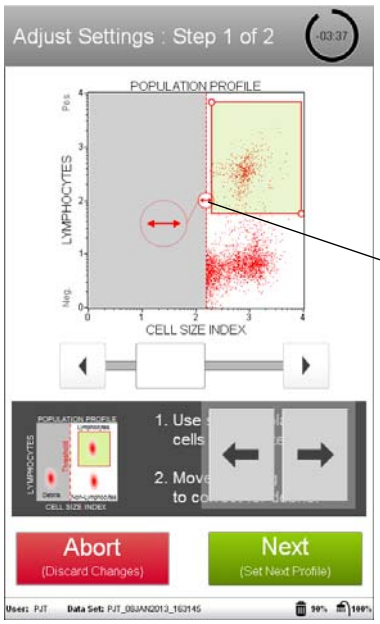
2. Select **Run Assay**.



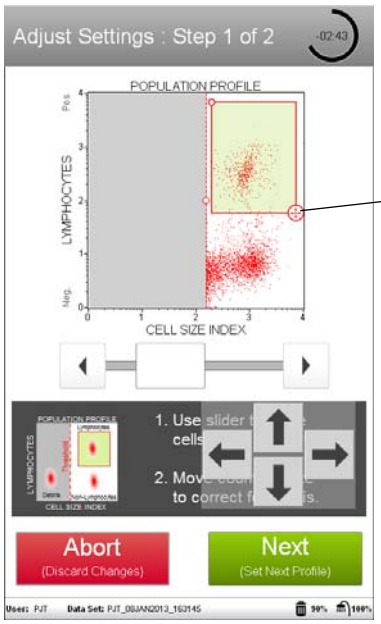
3. Adjust the instrument settings.
   - Load a stained sample for adjusting the settings and select **Run**. Perform the adjust settings steps using a stained sample.
   - Or, to retrieve previously saved instrument settings, select **Retrieve Settings**. For more information on retrieving settings, 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 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 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.



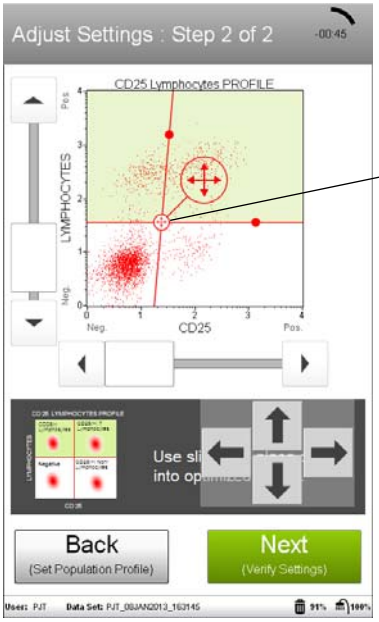
Touch threshold to move right/left.



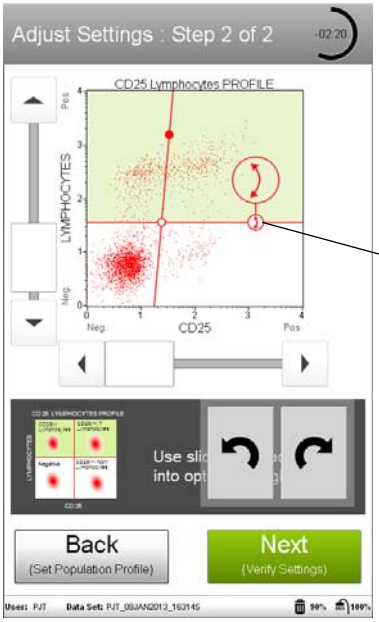
Touch and drag upper-left or lower-right corner to adjust the gate.

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 CD25 vs LYMPHOCYTES plot, if necessary.
   - Adjust the x and y-axis sliders to position the CD25(-) and CD25(+) 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.



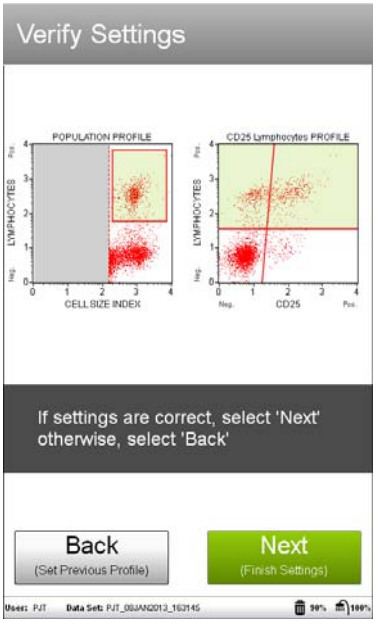
Touch center to move the fixed quadrant markers in any direction.



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**.

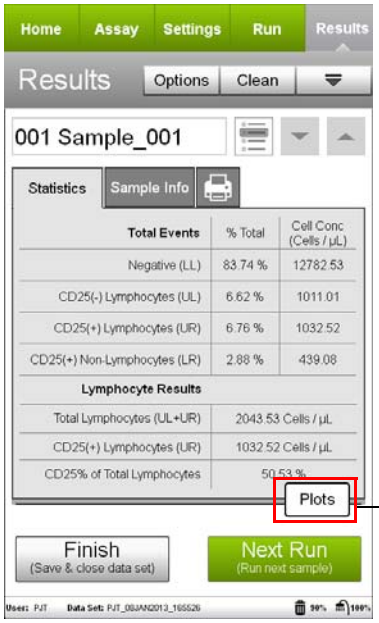


9. Enter the sample ID for the first sample by touching the field, then using the keypad to input the ID. Touch **Done** when you finish entering the ID. If necessary, change the **Events to Acquire** and/or **Dilution Factor** by touching the field, then selecting the value from the pop-up menu. Select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

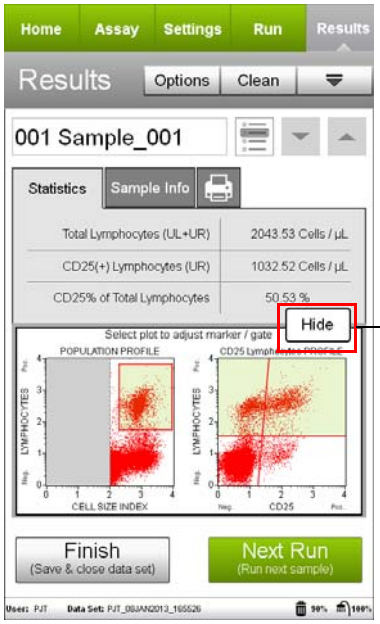
10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.

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.



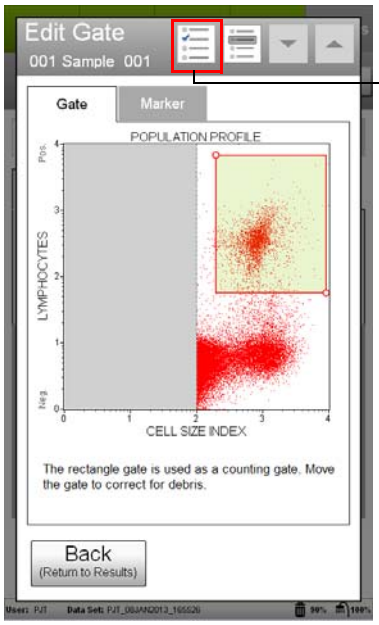
Select to display plots.



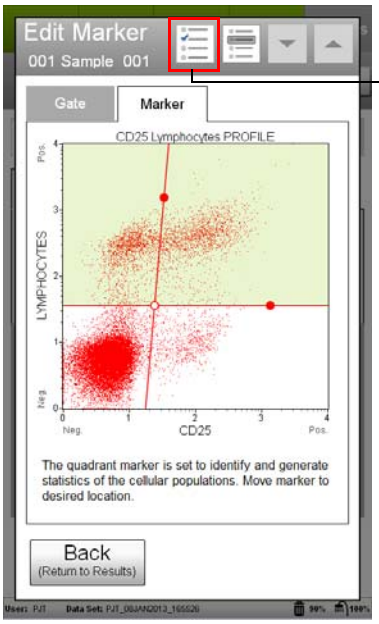
Select to hide plots.

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.

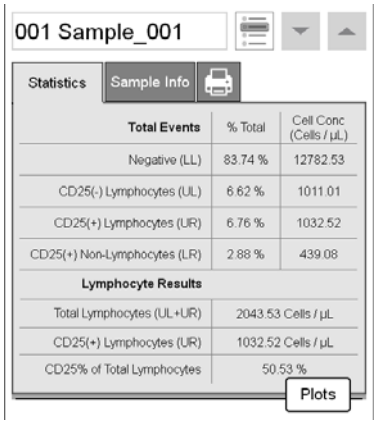
NOTE: During the run, a message may appear prompting you to load a tube of DI water for a Quick Clean. Load the water then select **Clean** to perform the Quick Clean. Select **Next** to continue with the run. The frequency of Quick Cleans was set by your system administrator. Your administrator may 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.

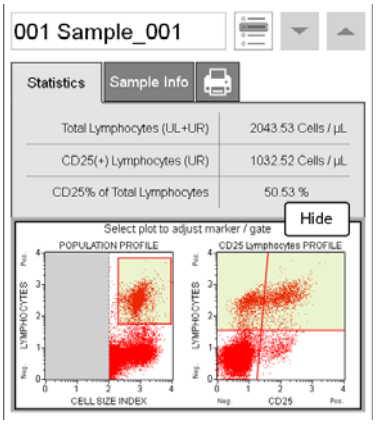
Results

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: Non-CD25 lymphocytes [lymphocyte(+) and CD25(-)]
  upper-right: CD25(+)lymphocytes [lymphocyte(+) and CD25(+)]
  lower-left: Negative cells [lymphocyte(-) and CD25(-)]
  lower-right: CD25(+) non-lymphocytes [lymphocyte(-) and CD25(+)]
- concentration and percentage of cells in each quadrant
- CD25(+) lymphocyte concentration, total lymphocyte concentration, and CD25 percent of total lymphocytes
- dilution factor (input value)



A



B

Figures A and B. Whole blood was stained with the Muse™ Human Lymphocyte CD25 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/µL) for the events in each quadrant out of the total events. Additionally, the Lymphocyte Results show the total lymphocyte concentration, the CD25(+) lymphocyte concentration, and the CD25 percentage of total lymphocytes. The first plot in Figure B shows Cell Size Index vs Lymphocytes and a lymphocyte gate, and the second plot shows CD25 vs Lymphocytes.

Technical Tips

1. All kit reagents 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 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 rate decreases dramatically; instrument clogging; too many cells	• 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. • 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.
Too few cells	• Restain sample using 180 µL of Human 1X Lysing Solution. Ensure dilution factor in the software is set to 20. • If using PBMCs, ensure that enough cells were stained as described in "Staining Protocol" on page 5. Restain sample using a more concentrated stock cell suspension.
Background staining and/ or non-specific staining of cells	• Ensure sample was mixed thoroughly prior to acquiring. • Ensure dried blood from side of tube was removed prior to staining. Unstained blood will contribute to erroneous results.
Low level of staining	• Ensure sample was stained for a minimum of 10 minutes followed by complete lysis for 15 minutes. • Treated donors may show less separation of cellular populations. Ensure a control sample is run.
Cells do not show shift in CD25 expression	• Cells may not have undergone activation or they may still be in the early activation stage. Positive control samples are recommended for each experiment. Positive controls should be appropriate for comparison with the test procedure or test cell population. Treatments to induce activation include, but are not limited to PHA and PMA/ionomycin. A time course of activation may need to be performed for different compounds to determine optimal detection conditions. • Although the assay procedure has been optimized to function utilizing both lysed whole blood and PBMCs, every donor may respond differently. A lack of signal may indicate that excess antibody will need to be used during the staining procedure or that the staining time needs to be increased.
Samples appear to be activated when low level of activation is expected	Samples may be compromised. Negative controls should be a sample not treated to induce activation.

Potential Problem	Experimental Suggestions
Variability in day-to-day and sample-to-sample 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. • Ensure that samples are lysed for at least 15 minutes with room temperature Human 1X Lysing Solution. • 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.

References

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Related Kits

- Muse™ Human CD4 T Cell Kit - MIM100101
- Muse™ Human CD8 T Cell Kit - MIM100102
- Muse™ Human B Cell Kit - MIM100103
- Muse™ Human Lymphocyte CD69 Kit - MIM100104
- Muse™ System Check Kit - MCH100101
- Muse™ Count & Viability Kit (100T) - MCH100102
- Muse™ Annexin V & Dead Cell Kit - MCH100105

Warranty

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