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Muse® Nitric Oxide Kit User's Guide

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For Research Use Only. Not for use in diagnostic procedures.
4600-3451, Rev C
Catalog No. MCH100112 (100 tests)
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Application

The Muse® Nitric Oxide Kit allows for the quantitative simultaneous measurements of two important cell health parameters—changes in intracellular nitric oxide activity levels and cellular plasma membrane permeabilization or cell death. Minimal sample preparation is required in this no-wash, assay to obtain accurate and precise results on both suspension and adherent cells.

The software provides the percentage and concentration of live cells, live cells with nitric oxide activity, dead cells with nitric oxide activity, dead cells, and the total nitric oxide-positive cells (live and dead).

The Muse Nitric Oxide Kit is for use with the Guava® Muse Cell Analyzer. The Muse System makes sophisticated fluorescent-based analysis fast, easy, convenient, and affordable. After loading samples onto the Guava Muse Cell Analyzer, intuitive software provides detailed or summary analysis of your cell sample in a few short steps.

Sufficient reagent is provided for the preparation and analysis of 100 tests.

For Research Use Only. Not for use in diagnostic procedures.

Test Principle

Nitric oxide (NO) is a small, gaseous, reactive molecule generated in a wide variety of cells as a product of the conversion of L-arginine into L-citrulline by the enzyme nitric oxide synthase (NOS).[1] NO is known to be involved in a variety of biological functions and activities ranging from vasodilation, neurotransmission, and anti-microbial and anti-tumor activities.[2] The constitutive isoform of NOS, found in neurons and endothelial cells, produces very low amounts of nitric oxide in a calcium- and calmodulin-dependent fashion.[3] The inducible isoform, found in macrophages, fibroblasts, and hepatocytes, produces NO in relatively large amounts in response to inflammatory or mitogenic stimuli and acts in a host-defensive role through its oxidative toxicity.[4]

NO also acts as a transcellular messenger molecule in many key physiological and pathological processes ranging from inflammation to apoptosis.[5] NO activates the apoptotic signal cascade in some situations, whereas it protects cells against spontaneous or induced apoptosis in other cases.[6-10] NO has been known to modulate different cancer-related events including angiogenesis, apoptosis, cell cycle, invasion, and metastasis.[11-12] The intracellular detection of nitric oxide is important for both mechanistic and biomedical research.

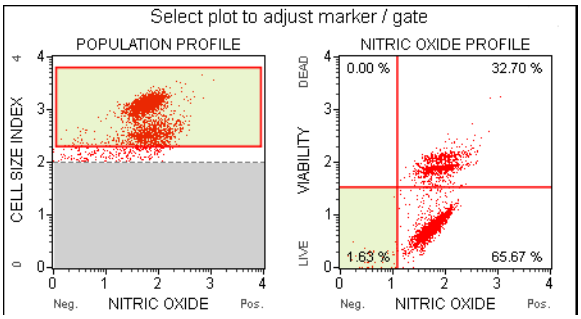
The Muse® Nitric Oxide Kit can simultaneously monitor the count and percentage of cells exhibiting nitric oxide activity and cell death. The kit consists of the Muse Nitric Oxide Reagent and the dead cell dye 7-AAD.

- The Muse Nitric Oxide Reagent is a membrane permeable novel reagent DAX-J2 Orange that generates a highly fluorescent product upon NO oxidation inside the cell. The reagent is non-toxic and does not depend on the action of cellular esterases for reactivity and is photostable.
- A dead cell marker (7-AAD) is also included in the kit as an indicator of cell membrane structural integrity and cell death. It is excluded from live cells with intact membranes, but permeates membranes that are compromised or stressed as well as dead cells. It exhibits increased fluorescence in the viability parameter.

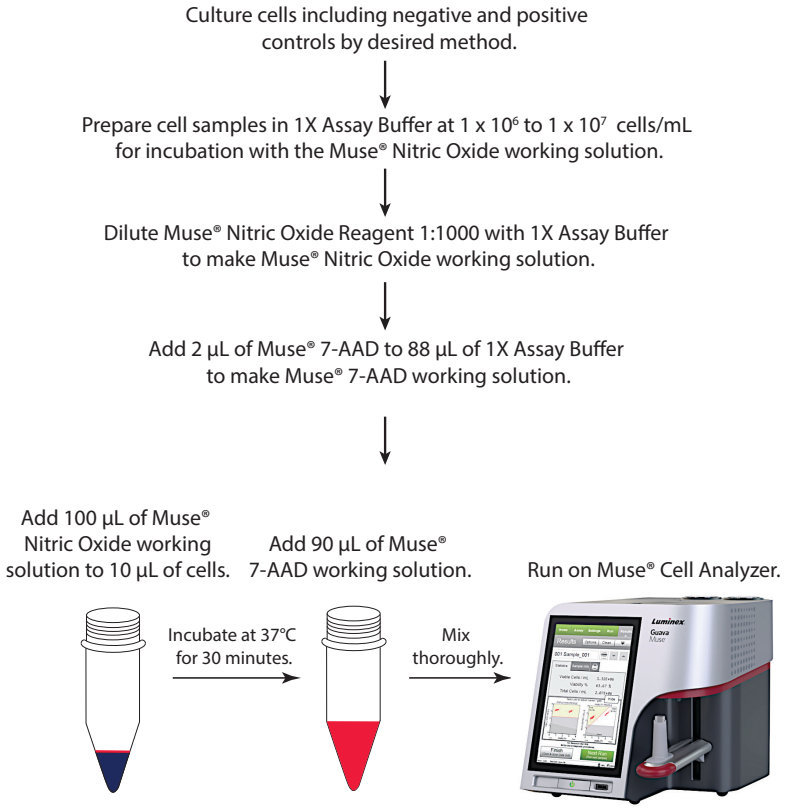
Four populations of cells can be distinguished in the assay:

- Live Cells with no Nitric Oxide Activity (negative): Nitric Oxide(-) and 7-AAD (-)
- Live Cells with Nitric Oxide Activity: Nitric Oxide(+) and 7-AAD(-)
- Dead Cells with Nitric Oxide Activity: Nitric Oxide(+) and 7-AAD(+)
- Dead Cells with no Nitric Oxide Activity: Nitric Oxide(-) and 7-AAD(+)

Figure 1: Representative plots from the Muse Nitric Oxide Assay. The first plot has a threshold marker for eliminating debris based on cell size, as well as a gate for gating on cells. The second plot shows gated cells with quadrant markers providing data on four cell populations—live with no nitric oxide activity, live with nitric oxide activity, dead with nitric oxide activity, and dead with no nitric oxide activity.



Summary of Protocol



Kit Components

- Muse® Nitric Oxide Reagent (Part No. 4700-1666, 100 tests/bottle)
- Muse 7-AAD (Part No. 4700-1673, 100 tests/bottle)
- 1X Assay Buffer (Part No. 4700-1330, 100 mL)

Materials Required but Not Supplied

- Guava® Muse® Cell Analyzer
- Cell suspension, treated and untreated to undergo cellular stress
- Micropipettors
- Disposable micropipettor tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR Catalog No. 16466-030, or equivalent)
- Vortex mixer
- 100% bleach solution
- Disposable gloves
- Deionized water
- Muse System Check Kit (Catalog No. MCH100101)
- Muse Cell Dispersal Kit (Catalog No. MCH100107), optional
- Muse Count & Viability Kit (Catalog No. MCH100102 [100T] or Catalog No. MCH600103 [600T]), optional
- Guava® ICF Instrument Cleaning Fluid (Catalog No. 4200-0140), optional

Precautions

- The Muse® Nitric Oxide Kit is intended for research use only.
- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
- The Muse Nitric Oxide Reagent and Muse 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 Safety Data Sheet (SDS) sheet for specific information on hazardous materials.
- Avoid microbial contamination of the solution, which may cause erroneous results.
- All biological specimens and materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
- Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous result.

- 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 Muse Nitric Oxide Reagent and Muse 7-AAD contain light-sensitive dyes. Store in the dark at –20°C and shield from excessive exposure to light during use.
- 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.
- Safety Data Sheets (SDSs) for kit reagents are available from our website (www.luminexcorp.com) or by contacting Luminex Technical Support.

Storage

- Store the Muse® Nitric Oxide Reagent frozen at –15° to -25°C, desiccated and protected from light. For long term storage, divide the stock solution into small aliquots to minimize the freeze-thaw cycles. When stored at –20°C, desiccated and protected from light, these aliquots are stable for at least 6 months.
- Store the 1X Assay Buffer and Muse 7-AAD refrigerated at 2° to 8°C. Do not freeze.

Before You Begin

We highly recommend that the 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® Nitric Oxide Kit, the stability of individual cell types may vary.

Time considerations: The process of staining cells with the Muse Nitric Oxide Kit using the recommended protocol below takes 30 to 40 minutes. Acquiring your data on your Guava® Muse Cell Analyzer takes less than 3 minutes per sample, depending on the concentration and desired number of events to acquire. However, preparing your cells for testing may require periodic maintenance and cultivation. Once you cultivate the proper number of cells for the experiment, it takes and additional 2 to 48 hours of culture with various inducers to stimulate detectable nitric oxide expression.

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

Allow the Muse® Nitric Oxide Reagent, Muse 7-AAD, and 1X Assay Buffer to warm to room temperature, protected from light.

Muse® Nitric Oxide Reagent Working Solution

Prepare Muse® Nitric Oxide working solution by diluting the Muse Nitric Oxide Reagent stock solution 1:1000 in 1X Assay Buffer. Each sample tested requires 100 µL of Muse Nitric Oxide Reagent working solution. The Muse Nitric Oxide Reagent working solution must be prepared immediately before use. Store at room temperature, protected from light until use.

1. Dilute the stock solution as follows:

   NOTE: Quantities below include one or more extra tests to allow for sufficient volumes.

Stock solution / buffer	10 tests	50 tests	100 tests
Muse Nitric Oxide Reagent	1 µL	5 µL	10 µL
1X Assay Buffer	999 µL	4995 µL	9990 µL

Muse® 7-AAD Working Solution

Prepare Muse® 7-AAD working solution by diluting the Muse 7-AAD stock solution 1:45 in 1X Assay Buffer. Each sample tested requires 90 µL of Muse 7-AAD working solution. The Muse 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.

1. Dilute the Muse 7-AAD stock solution with 1X Assay Buffer as suggested in the following table:

   NOTE: Quantities below include one or more extra tests to allow for sufficient volumes.

Stock solution / buffer	1 test	10 tests	100 tests
Muse 7-AAD	2 µL	20 µL	200 µL
1X Assay Buffer	88 µL	880 µL	8800 µL

Staining Protocol

For instructions on preparing cell suspension, see "Appendix A: Cell Sample Preparation" on page 15. Make sure to stain positive and negative controls.

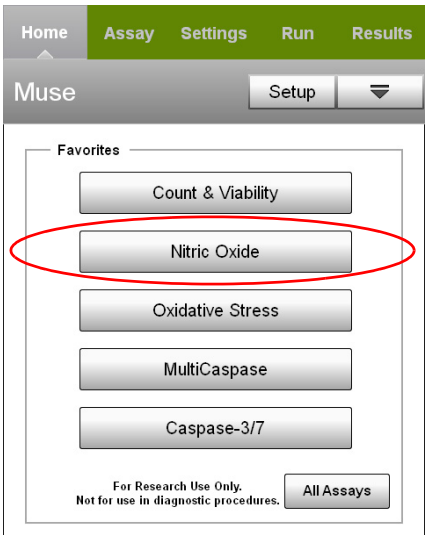
1. Prepare cell samples in 1X Assay Buffer at 1 x 10^6 to 1 x 10^7 cells/mL.
2. Add 10 µL of cells in suspension into each tube.
3. Add 100 µL of Muse® Nitric Oxide Reagent working solution to each tube.
4. Mix thoroughly by pipetting up and down or vortexing at medium speed for 3 to 5 seconds. Loosely cap the tube.
5. Incubate samples for 30 minutes in the 37°C incubator with 5% CO_2.
6. After incubation, add 90 µL of Muse 7-AAD working solution to each tube.
7. Mix thoroughly by pipetting up and down or vortexing at medium speed for 3 to 5 seconds.
8. Incubate at room temperature for 5 minutes, protected from light.

Samples are ready to run on the Guava® Muse Cell Analyzer.

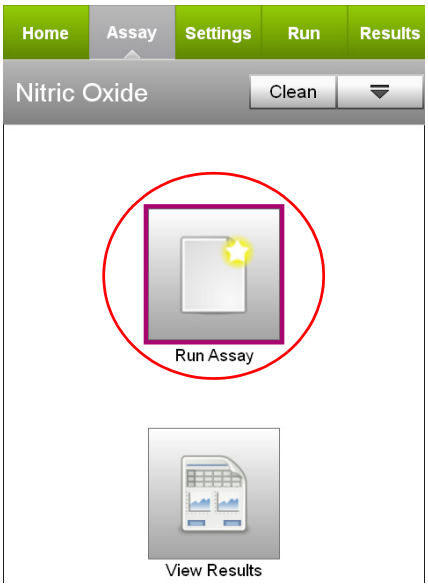
Setup and Acquisition on the Guava® Muse® Cell Analyzer

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 **Nitric Oxide** from the main menu.



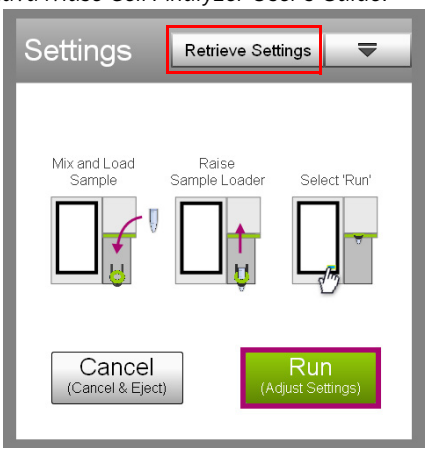
2. Select **Run Assay**.



3. Adjust the instrument settings.
   - Load a stained sample for adjusting the settings and select **Run**.

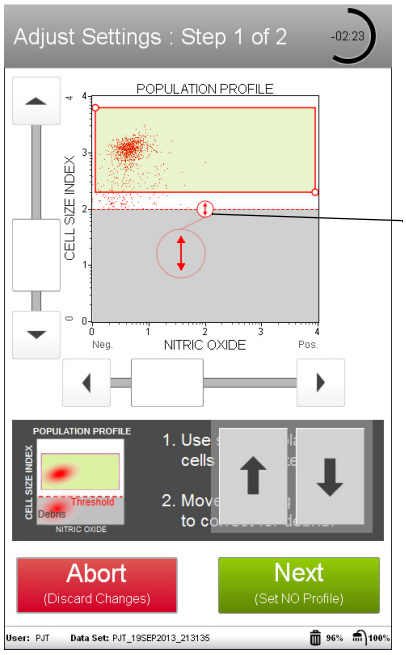
NOTE: Perform the adjust settings step using a negative control, then verify the settings using a positive control.

- 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*.

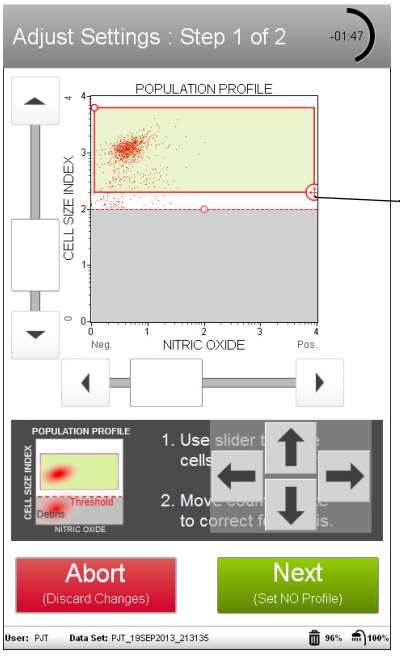


4. Fine tune the settings for the NITRIC OXIDE vs. CELL SIZE INDEX plot, if necessary.
   - Adjust the CELL SIZE INDEX slider to the left of the plot to move the cell population into the gate (green region).
   - Drag the threshold to exclude any cellular debris. Touch the threshold and drag to make 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 adjust settings step times out (after 2 minutes), remove the tube and mix well before reloading. 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.

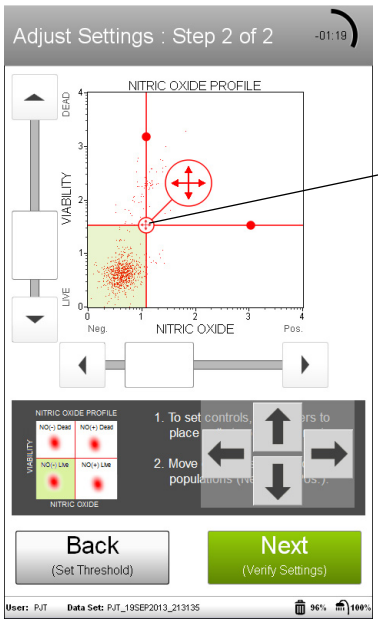


Touch and drag upper-left or lower-right corner to adjust the gate.

This example data shows typical gate and marker settings.

5. Select **Next** when the adjustments are complete.

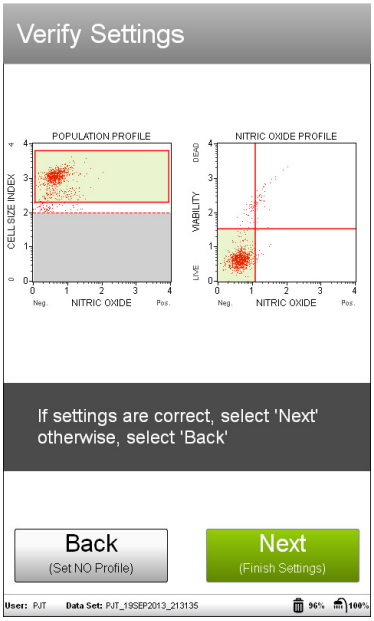
6. Fine tune the Nitric Oxide vs. Viability plot, if necessary.
   - Adjust the Viability slider to place all populations on scale.
   - Adjust the quadrant markers, if necessary. You can move the marker intersection in any direction, as well as adjust the angle of each line. To move the markers as they 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.



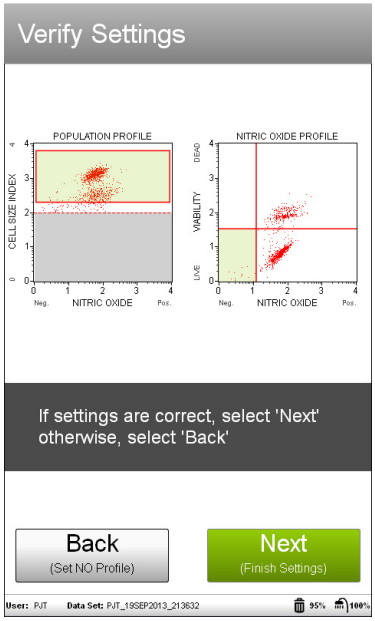
Touch center to move the fixed quadrant markers in any direction.

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

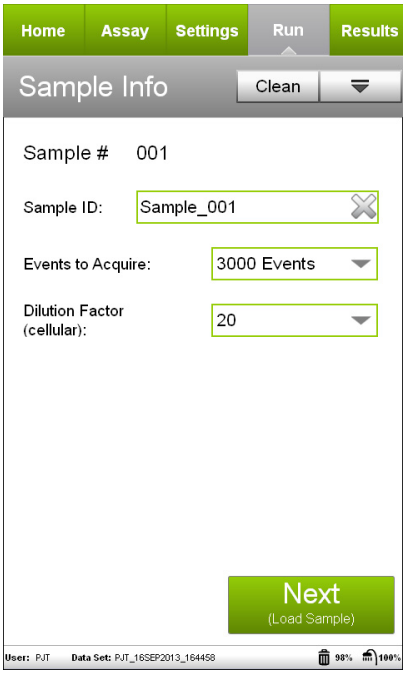
Negative control



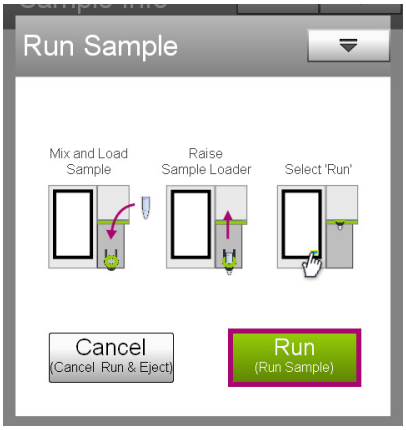
Positive control



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**.

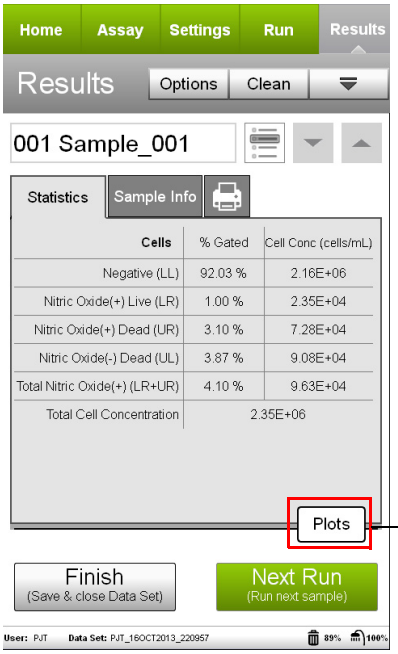


10. Mix the first sample and load it on the instrument. Select **Run** to run the sample.

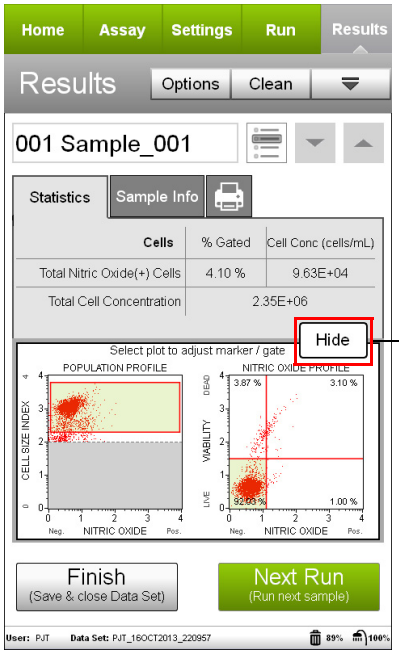


11. When acquisition is complete, the results are displayed. If desired select **Plots** to display dot plots for the sample.

You can view or change the sample ID, 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.



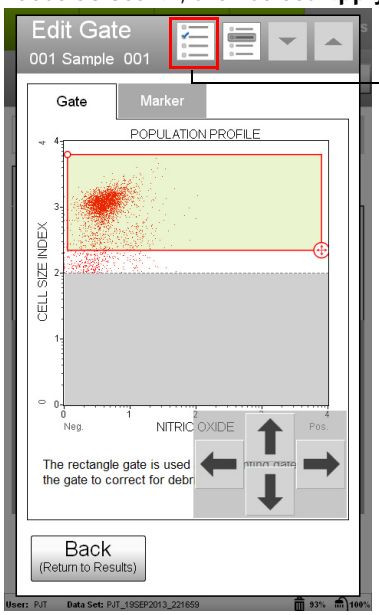
Select to display plots.



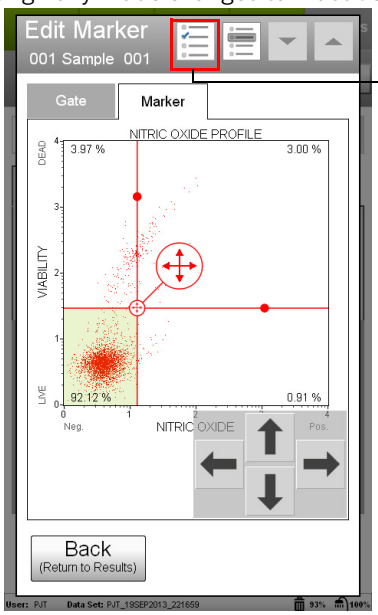
Select to hide plots.

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



Apply changes to other samples.

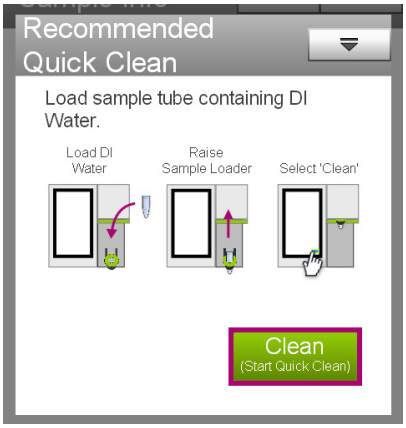


Select to apply changes to other samples.

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.



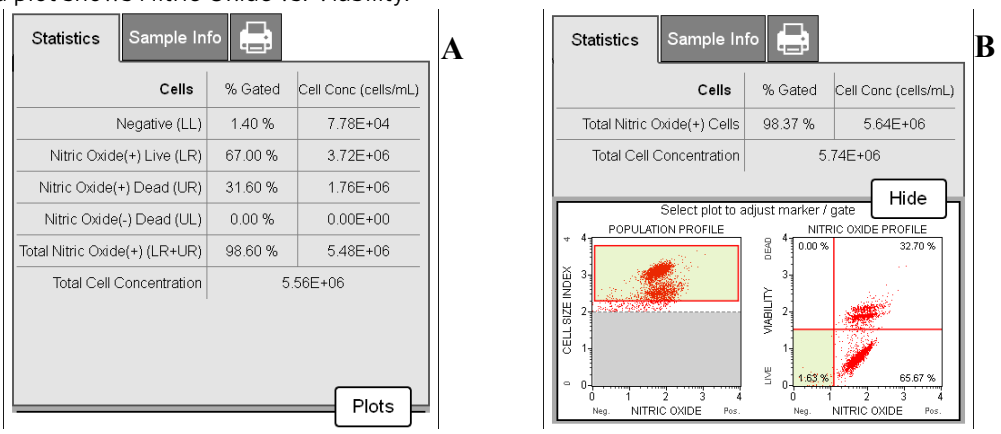
14. When you have acquired the last sample, select **Finish**, enter a name for the data set or leave the default, and select **OK**.
15. (Optional) Select **Options** in the title bar to rename the data set, export the data set, save the current instrument settings, or view the event log. Refer to the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

- sample number
- sample ID
- Percent and concentration (cells/mL) of gated cells in each quadrant
  - Lower-left: live cells with no nitric oxide activity [Nitric Oxide(-) and Dead cell marker (-)]
  - Lower-right: live cells with nitric oxide activity [Nitric Oxide(+) and Dead cell marker (-)]
  - Upper-right: dead cells with nitric oxide activity [Nitric Oxide(+) and Dead cell marker (+)]
  - Upper-left: dead cells with no nitric oxide activity [Nitric Oxide(-) and Dead cell marker (+)]
- Concentration and percentage of total cells with nitric oxide activity (cells in upper-right and lower-right quadrants)
- Dilution factor (input value)
- Fluorescent intensity values for negative and Nitric Oxide(+) cell populations

Figures A and B: RAW264.7 cells were treated with 100 nM LPS and 100 U/mL mouse IFNg for 22 hours to induce nitric oxide activity, then stained with the Muse® Nitric Oxide 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 nitric oxide+ cells. The first plot in Figure B shows Nitric Oxide vs. Cell Size Index and the second plot shows Nitric Oxide 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 cellular breakdown and splashing, resulting in volume loss and erroneous results.
2. The default number of events to acquire is 3000. You may select a different number; however, your statistical error will increase as you decrease the number of acquisition events.
3. If results deviate from expected values, check that the correct value was entered for the dilution factor. You can recalculate the cell counts by opening the data file and reentering the correct dilution factor for the specific sample. Cells counts will be recalculated automatically.
4. 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.
5. If you are acquiring 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 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 Luminex Technical Support for help.
6. Muse Nitric Oxide Kit 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 kit with a "clumpy" cell type, we recommend using Muse Cell Dispersal Kit (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.

Troubleshooting

Potential Problem	Experimental Suggestions
Acquisition taking longer than expected or progress bar stops during acquisition	Ensure that the Muse® System Check (Part No. MCH100101) procedure was run and passed. If the progress bar stops during acquisition, the fluid system may be clogged. Run a Quick Clean procedure. It can be performed during or after an assay. This will wash away any material forming within the glass capillary walls.
Instrument clogging; too many cells	• Cell concentration too high. Decrease the number of cells per microliter by diluting the sample to 300–500 cells/µL. The Guava® Muse Cell Analyzer give the most accurate data when the flow rate is less than 500 cells/µL. • 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.
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 to adjust the cell concentration below 500 cells/µL. For best results, we recommend that the cell concentration is in the range of 200 to 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 samples 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 nitric oxide activity or treated with a specific nitric oxide inhibitor. Typically, negative control samples show a low level of nitric oxide and/or dead cell marker positive cells, distinct from that of induced cells, because healthy cell cultures contain a small number of stressed and /or dead cell. However, sub-optimal culture conditions may stress cells in culture, causing them to exhibit nitric oxide activity in the absence of an experimental induction treatment. The negative control from a stressed culture shows increased nitric oxide and/or dead cell marker reactivity.

Potential Problem	Experimental Suggestions
Low level of staining	• If there is a low level of nitric oxide staining, the Muse Nitric Oxide Reagent may have been degraded. Verify proper storage and handling of the reagent. Prepare fresh working solution immediately before staining cells. • If there are no nitric oxide–positive cells, the cells may not be fully induced or the Muse Nitric Oxide Reagent may not have been taken up correctly by the cells. The nitric oxide staining results can vary over time as cellular stress progresses. To determine optimal induction, conduct a time-course study to achieve the best results for nitric oxide 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 nitric oxide activity. Treatments used to induce cellular stress in various cell lines include: serum starvation, activation of macrophages with lipopolysaccharide and IFNδ, UV irradiation, and treatment with a compound known to induce nitric oxide activity in your cell line.
Poor separation of negative and nitric oxide(+) populations	• If the separation between populations is poor, the Muse Nitric Oxide Reagent concentration may be too low. Muse Nitric Oxide Reagent has been formulated for optimal performance using RAW264.7 and 143B cells. Other cells may show different patterns of reactivity that require adjustments to the amount of reagent used. For best results, titer the Muse Nitric Oxide Reagent to determine the optimal amount for maximal staining of cells. • If the separation between populations is dimmer than expected, it is possible that the stained samples or Muse Nitric Oxide reagent may have been exposed to light or gone through repeated freeze-thaw cycles. Repeat the staining using fresh reagents and avoid prolonged exposure of reagents and stained samples to light.
Percent of nitric oxide(+) cells increases over time	If the percent of nitric oxide(+) 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 Muse Nitric Oxide 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 between cell lines, and extended storage of stained samples may adversely affect results. We recommend acquiring samples within 2 hours after sample 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 experimental cell cultures to ensure that cell viability and cell numbers being analyzed are consistent. Any drop in cell numbers or viability can influence experimental results. • If there appears to be day-to-day variation of the staining pattern, ensure the Guava Muse Cell Analyzer is working properly. Run the Muse System Check (Part No. MCH100101) 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 nitric oxide species content. The amount of nitric oxide activity expressed intracellularly varies among cell types after cellular stress or apoptosis is induced.
- The Muse® Nitric Oxide Kit is designed for use on unfixed cells. Fixing cells may yield inaccurate results.
- The Guava® Muse Cell Analyzer and Muse Nitric Oxide Kit yield optimal results when the stained cell sample used for acquisition is between 5 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 throughput, we recommend using between 1 x 10^5 to 5 x 10^5 cells/mL when possible.

Appendix A: Cell Sample Preparation

The following protocols describe how to harvest non-adherent and adherent cells cultured in 96-well plates or in flasks or other tissue culture vessels.

Preparing Non-adherent Cells

1. Set up initial culture conditions, such that after culture and treatment cells are resuspended at a concentration of 1 x 10^6 to 1 x 10^7 cells/mL (1 x 10^4 to 1 x 10^5 cells/sample) in 1X Assay Buffer.
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. Wash cells to remove the dissociating reagent. Resuspend the cells in 1X Assay Buffer so that the final concentration is between 1 x 10^6 to 1 x 10^7 cells/mL (1 x 10^4 to 1 x 10^5 cells/sample).
3. Proceed to "Staining Protocol" on page 5.

References

1. Boucher JL, Moali C, Tenu JP. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for L-arginine utilization. *Cell Mol Life Sci*. 1999;55:1015-1028.
2. Omer N, Rohilla A, Rohilla S, Kushnoor A. Nitric Oxide: Role in Human Biology. *Int J Pharma Sci Drug Res*. 2012;4:105-109.
3. Bredt DS. Endogenous nitric oxide synthesis: biological functions and pathophysiology. *Free Radic Res*. 1999;31:577-596.

4. Bogdan, C. Nitric oxide and the immune response. *Nat Immunol*. 2001;2:907-915.
5. Guzik, T, Korbut R, Adamek-Guzik T. Nitric oxide and superoxide in inflammation and immune regulation. *J Physiol Pharmacol*. 2003;54:469-487.
6. Brune B, Sandau K, Knetten A. Apoptotic cell death and nitric oxide: activating an antagonistic transduction pathways. *Biochemistry*. 1998;63:966-975.
7. Choi BM, Pae HO, Jang SI, Kim YM, Chung HT. Nitric Oxide as a pro-apoptotic as well as anti-apoptotic modulator. *J Biochem Mol Biol*. 2002;35:116-126.
8. Kim PK, Zamora R, Petrosko P, Billiar TR. The regulatory role of nitric oxide in apoptosis. *Int Immunopharmacol*. 2001;1:1421-1441.
9. Weller R, Billiar T, Vodovotz Y. Pro- and anti- apoptotic effect s of nitric oxide in irradiated keratinocytes: the role of superoxide. *Skin Pharmacol Appl Skin Physiol*. 2002;15:348-352.
10. Ying L, Hofseth LJ. An emerging role for endothelial nitric oxide synthase in chronic inflammation and cancer. *Cancer Res*. 2007;67:1407-1410.
11. Li CQ, Pang B, Kiziltepe T, Trudel LJ, et al. Threshold effects of nitric oxide-induced toxicity and cellular responses in wild-type and p53-null human lymphoblastoid cells. *Chem Res Toxicol*. 2006;19:399-406.
12. Umansky V, Schirrmacher V. Nitric oxide-induced apoptosis in tumour cells. *Adv Cancer Res*. 2001;82:107-131.

Related Kits

- Muse® System Check Kit – MCH100101
- Muse® Count & Viability Kit (40mL) – MCH100102
- Muse® Annexin V and Dead Cell Kit – MCH100105
- Muse® Cell Dispersal Reagent Kit – MCH100107
- Muse® Caspase-3/7 Kit – MCH100108
- Muse® MultiCaspase Kit – MCH100109
- Muse® MitoPotential Kit – MCH100110
- Muse® Oxidative Stress Kit – MCH100111
- Muse® Ki67 Proliferation Kit – MCH100114