



4600-0060
rev B

Guava Express™ 7-AAD

For Viability Determination in Protein Expression Analysis

Research Use Only

1. PRODUCT DESCRIPTION AND INTENDED USE _____

The Guava Express™ application is used to evaluate the binding activity of primary antibody reagents as direct-labeled conjugates or as indirect conjugates when used with Guava Express secondary reagents. Guava Express can be used for titrating purified monoclonal antibody preparations, assessing target activity of antibody clones, and identifying cell populations that express a specific protein marker.

Cells from a variety of sources may be assayed in Guava Express, including in vitro cultured cells and peripheral blood leukocytes. A standard assay for Guava Express uses 1×10^5 cells in a 50 μ L staining reaction. Assay results may be affected by the nonspecific uptake or binding of staining reagents by dead cells. To identify these events, the cell impermeant nucleic acid dye, 7-aminoactinomycin D (7-AAD) can be included in a Guava Express assay as an indicator of cell viability. 7-AAD is excluded from live, healthy cells but permeates dead and dying cells. Guava Express 7-AAD is provided as a convenient, ready-to-use solution, for use with the Guava Express phycoerythrin (PE)-conjugated secondary reagents.

Guava Express data are displayed on a dot plot and two histogram plots. The PE-conjugated secondary reagent fluorescence appears in the PM1 parameter and 7-AAD appears in the PM2 parameter. Dead cells (7-AAD positive cells) may be excluded from the analysis by electronic gating of the acquired events. Results are exported to a Microsoft Excel file automatically. Data files can be reviewed and reanalyzed using CytoAnalysis software or an FCS 3.0-compatible program if the option to save FCS files was selected.

2. MATERIALS PROVIDED _____

- Guava Express 7-AAD (Catalog No. 4000-0061, 1.0 mL)

3. HANDLING AND STORAGE _____

1. Store the Guava Express reagents refrigerated (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.
2. Guava Express 7-AAD is light-sensitive. Shield from excessive exposure to light.

4. WARNINGS AND PRECAUTIONS _____

1. The Guava Express reagents are intended for research use only.
2. Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this reagent.
3. Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic and mutagenic reagents.

4. The Guava Express reagents contain sodium azide, which is toxic. Contact with acids liberates toxic gas. Flush plumbing with copious amounts of water when disposing of azide compounds to avoid potentially explosive conditions arising from azide deposits in pipes.
5. Avoid microbial contamination, which may cause erroneous results.
6. 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.
7. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.

5. EQUIPMENT AND MATERIALS REQUIRED _____

- Guava™ Technologies Personal Cytometer (PC)
- Primary antibody
- Isotype control antibody (optional)
- Positive control primary antibody (optional)
- Guava Express Secondary Reagent (optional)
- Guava Express 7-AAD
- Cell suspension for primary antibody labeling
- Phosphate buffered saline (PBS), or equivalent balanced salt solution, pH 7.2 to 7.4, supplemented with 5 mM EDTA and 0.05% sodium azide. (1 to 3% bovine serum albumin (BSA) can be added if desired). Buffer should not contain phenol red indicator and should be kept cold during staining.
- Micropipettors
- Disposable micropipettor tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog No. 20170-215 or equivalent) OR
- Sample acquisition tubes, 1.2 mL titer tubes (E&K Scientific, Catalog No. 604508-RC or equivalent)
- Vortex mixer
- Centrifuge
- Disposable gloves
- 20% bleach solution
- Deionized water

6. BEFORE YOU BEGIN _____

1. Turn on the laptop computer.
2. After the computer startup is completed, turn on the Guava PC.
3. Start CytoSoft by double-clicking the **CytoSoft** application icon on the desktop.
4. When initialization is complete, select **Guava Express** from the main menu. Allow the system to warm up for at least 15 minutes before running the analysis.

7. REAGENT AND SAMPLE PREPARATION _____

The following procedure for staining with a directly-conjugated antibody is provided as a guideline. Procedures for indirect staining are provided with the Guava Express secondary reagents. Refer to the *Guava Personal Cytometer User's Guide* or contact Guava Technical Service for additional information. If you have a preferred procedure for antibody staining suitable for your application and cell types, prepare your samples accordingly.

Staining with Direct Conjugates and Express 7-AAD Reagent in Tubes

1. Prepare cell suspensions for antibody labeling and Express 7-AAD staining. The Guava Express reagents are designed to stain 1×10^5 cells per sample in a 50 μ L staining volume.

NOTE: Follow approved protocols for preparing suspensions from tissue samples or in vitro cultures, including methods for tissue fractionation, disruption, or release of adherent cells from culture substrates.

2. Pipet 1×10^5 cells into each microcentrifuge or sample acquisition tube per test. Include additional tubes for unstained, positive, and negative controls. A suggested negative control would be stained with an appropriate isotype control antibody to assess background level staining.

3. Prepare the primary and control antibody solutions in cold PBS buffer at concentrations to allow for the addition of 5 μ L of Express 7-AAD reagent and maintain a 50 μ L final staining volume per sample.

NOTE: You may need to titer the reagents to suit your specific assay conditions.

4. Add primary or control antibody to each sample to yield the desired final concentration of antibody (usually between 0.1 to 10 μ g/mL). Add PBS buffer to tubes for unstained cells and for 7-AAD only control.

5. Add 5 μ L of Express 7-AAD reagent to test samples and to appropriate control tubes.

6. Incubate for 30 minutes on ice or at 2 to 8°C.

7. Add 1 mL of cold PBS buffer to each tube. Mix by gently vortexing and pellet the cells by centrifugation at 300 x g for 5 minutes. Carefully remove the supernatant without disturbing the cell pellet.

8. Gently vortex each tube to disrupt the pellet and add 500 μ L of cold PBS buffer to each tube. Mix well. Samples are ready for data acquisition on the Guava PC.

8. DATA ACQUISITION AND ANALYSIS _____

Set Thresholds

1. Mix the unstained cell sample gently and load it onto the Guava PC.
2. Click **Adjust Settings**.

The Adjust Settings screen will appear. The system will automatically set a threshold using default settings. After the instrument completes the initial settings function, you will need to adjust the forward scatter gain to position the main cell population within the dynamic range of the plot. Increase the forward scatter threshold setting to exclude debris without excluding cellular events. Make any necessary adjustments to the flow rate and voltage settings for PM1 and PM2 at this time. Refer to the *Guava Personal Cytometer User's Guide* for instructions.

3. After you have made the desired changes to the instrument settings, click **Next Step** to reset the instrument. The display will return to the acquisition screen.

Enter Information

1. Create a new data file by clicking **New Data**.
2. Enter the file name for data storage.
3. Enter the number of events to be acquired (default value is 1000).

Acquire Sample

1. Gently mix the isotype control and load it onto the Guava PC. Typically, the isotype control is used to assess non-specific staining.
2. Click **Isotype Control**. When sample acquisition is finished, the results appear in the tables and on the histogram and dot plots. These results are

saved under the Sample ID name Isotype Control in the data and spreadsheet files.

3. Set your gates and markers, as desired. Refer to the *Guava Personal Cytometer User's Guide* for details. Fluorescence for the Guava Express PE-conjugated secondary antibody appears in the PM1 histogram. 7-AAD fluorescence appears in the PM2 histogram.
4. Mix the positive control sample and load it on the Guava PC.
5. Click **Positive Control**. When the sample acquisition is finished, the results appear on the histogram and dot plots. These results are saved under the Sample ID name Positive Control in the data and spreadsheet files. Make additional adjustments to the gates and markers as desired.
6. If you want to modify the instrument settings based on results obtained from the isotype control or positive control, you can click **Adjust Settings** again and make the desired changes.
Reacquire the isotype control and positive control samples as in steps 1 through 5. This will overwrite the previous data files for these samples. Adjust the gates and markers as needed.
7. Mix the unstained sample and load it on the Guava PC. Usually, the unstained sample should be the first sample to acquire after setting the instrument settings, gates, and markers (after acquiring the isotype and positive controls).
8. Enter a sample identification in the Sample ID box, if desired.
9. Click **Acquire Sample**. When the sample acquisition is finished, the analysis results appear in data tables, histogram, and dot plots.
10. Mix the first stained sample or additional controls and load it onto the Guava PC.
11. Enter a sample identification in the Sample ID box (optional).
12. Click **Acquire Sample**.

If you check the **Autostart Enabled** box, you do not need to click **Acquire Sample**. Sample acquisition will start automatically when the next tube is loaded, allowing a new sample tube to be placed into the carrier, then raised back into position for sampling.

The file number automatically advances to the next sample number on the Control Panel. If you need to rerun a sample, change the file number on the control panel to overwrite the previous data. This will maintain the file count for the samples. However, you will not be able to enter individual sample identifications in the Sample ID box when acquiring in this mode.

13. Repeat steps 10 through 12 for each sample. Refer to the *Guava Personal Cytometer User's Guide* for more information on loading samples and running the program.

For each sample acquired, the analysis data is automatically exported into Microsoft Excel.

14. To start a new worksheet, click **New Data Set**. This creates a new data file.

Exit

1. When all the samples have been acquired, run Quick Clean or Guava Clean.
2. Leave a tube of deionized water on the cytometer when you are finished. Leaving the bleach tube loaded on the cytometer for prolonged periods may cause damage to the flow cell. Refer to the *Guava Personal Cytometer User's Guide* for more information on cleaning and maintenance.
3. Exit the Guava Express application by clicking **Main Menu**.

9. EXPECTED RESULTS

The Guava Express application performs calculations automatically. The results are displayed on the computer screen after each sample is acquired. Acquired

data are displayed in two histogram plots: PM1, which shows fluorescence of the PE conjugated reagent, and PM2, which shows 7-AAD fluorescence.

Within each histogram, a movable cursor allows gating of events. The analysis results of each sample are automatically displayed at the end of each acquisition. These include the mean and median fluorescence intensities of the sample, along with the %CV of the mean fluorescence intensity for PM1 and PM2. In addition, the percentage of events falling within each gate is shown. All data and instrument settings are automatically saved to Excel spreadsheets for future reference. You can reanalyze data later using CytoAnalysis software. If you selected the option to save data in FCS file format, you can analyze the data with other FCS 3.0-compatible programs.

An example of staining results obtained using Guava Express 7-AAD is shown in Figure 1. Jurkat cells were washed with PBS and stained with Guava Express 7-AAD. The 7-AAD fluorescence histogram of acquired events with live cells (7-AAD negative) is shown in red and dead cells (7-AAD positive) is shown in green as differentiated by the marker.

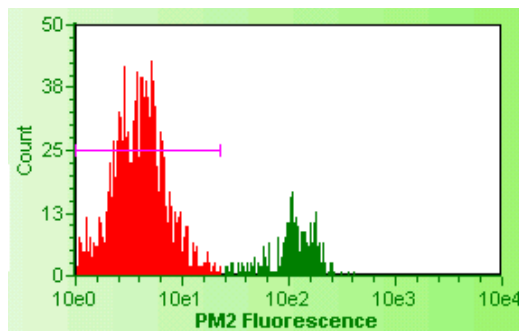


Figure 1 7-AAD fluorescence histogram of acquired events.

10. TROUBLESHOOTING TIPS

1. Mix each cell sample thoroughly on a vortex mixer before acquiring samples for consistent and accurate results.
2. If the concentration of the stained cell sample for data acquisition is high ($\geq 5 \times 10^5$ cells/mL), the Guava PC may not yield accurate results. Dilute the sample further with additional PBS buffer (to 1 mL volume) to bring the cell concentration into an acceptable range.
3. Acquire data on stained cells soon after staining. Dilution of the reagents slows, but does not stop, continued staining of the cells. Prolonged exposure to 7-AAD may result in a higher background fluorescence. If acquisition is to be delayed beyond 60 minutes, wash the stained cell suspensions using cold PBS buffer. Keep washed samples chilled and protected from light until data acquisition begins.
4. If the background fluorescence seems high after staining, pellet the cells by centrifugation at 300 x g, carefully remove the supernatant and resuspend the pellet in 0.5 to 1 mL of PBS buffer. Reacquire the data from the sample. An additional wash with PBS buffer may reduce the background fluorescence further.
5. Run the Guava Check application using the Guava Check kit (Catalog No. 4500-0020) to verify proper instrument function and accuracy.
6. Always run Guava Clean or Quick Clean with 20% bleach and deionized water tubes after using the ViaCount Reagent and before running Guava Express. Residual ViaCount Reagent may carry over into samples, affecting the results.

7. Periodically run Quick Clean using a deionized water tube (after every 20 to 25 sample acquisitions) to prevent a buildup from cell debris in the flow system. If your samples contain significant amounts of cellular debris, run Quick Clean more often to prevent clogs or blockage.
8. A clog or blockage of the flow system can be caused by cell aggregates, cell debris, bleach crystals, or other particulates. If you are acquiring data from a sample but the Cell Count number is not increasing and the Events to Acquire bar is not moving, there is probably a blockage of the flow system. Load a 20% bleach tube and click **Purge** to flush out the clog. Load a deionized water tube and run Quick Clean to remove bleach residue. If this procedure does not alleviate the problem, consult the *Guava Personal Cytometer User's Guide* or contact Guava Technical Service for additional help.

For more troubleshooting tips, refer to the *Guava Personal Cytometer User's Guide*.

11. LIMITATIONS

1. The Guava PC will yield optimal results when the stained cell sample for acquisition is between 1×10^4 to 5×10^5 cells/mL. To obtain the most accurate assay results, adjust the concentration of the cell samples to within the recommended range.
2. Guava Express reagents are formulated to meet most assay requirements. Modification of the staining protocol and reagent concentration(s) may be necessary to ensure optimal performance for individual assays.

12. DISCLAIMER OF WARRANTY

The product sold hereunder is warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. The sole liability for Guava Technologies, Inc. is limited to either replacement of the products or refund of the purchase price. Guava Technologies, Inc. is not liable for property damage, personal injury, or economic loss caused by the use of this product.

13. RETURN GOODS POLICY

Please inspect package(s) upon receipt and inform us immediately of any shortage or shipping errors. Claims must be made within 10 business days. Call our Customer Service department so they can authorize a return and provide shipping instructions.

14. TRADEMARKS AND PATENTS

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To order the Guava Personal Cytometer, call toll-free:

(866) 448-2827 or visit:
www.guavatechnologies.com.

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