



# Guava<sup>®</sup> ViaCount<sup>™</sup> Flex Reagent Package Insert

**RUO** For Research Use Only. Not for use in diagnostic procedures.  
4600-0530, Rev H  
Catalog No. 4700-0060 (500 tests) and 4500-0110 (100 tests)  
06/2020

## Technical Support

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## Description

The Guava® ViaCount™ Flex Reagent provides absolute cell counts and viability determinations on cell suspensions from a variety of mammalian and non-mammalian cell types, including adherent cells and insect cells such as SF-9. The ViaCount Flex Assay distinguishes between viable and non-viable cells based on the differential permeability of DNA-binding dyes in the ViaCount Flex Reagent. The fluorescence of each dye is resolved operationally to allow the quantitative assessment of both viable and non-viable cells present in suspension. The Guava ViaCount Flex Assay is intended as a rapid and reliable method for determining cell concentrations and viability when using 96-well microplates on the automated, high-throughput (HT) Guava Systems.

The ViaCount Flex Reagent contains two fluorescent DNA-binding dyes in a highly concentrated formulation of DMSO. It is thus optimized for use in 96-well microplates. This more concentrated formulation allows you to add the ViaCount Flex Reagent directly to the 96-well microplate containing cells, providing cell count and viability assessments with cell concentrations as low as  $1 \times 10^4$  cells/mL and with minimal sample preparation. You can also use the ViaCount Flex Reagent with a medium that is compatible with your specific cell line or for cell lines that stain less efficiently and may require a higher concentration of dyes.

ViaCount Flex Reagent allows for the rapid, reliable, and convenient assessment of absolute cell concentrations and viability of cell samples in 96-well microplates.

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## Materials Provided

- 4700-0060- Guava ViaCount Flex Kit
  - 0.2mLx 5 (4000-0130) ViaCount Flex Reagent
- 4500-0110- Guava ViaCount Flex Reagent
  - 0.2 mLx1 (4000-0130) ViaCount Flex Reagent
  - 0.5mL (4300-0160) Anhydrous DMSO

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## Materials Required but Not Provided

- Guava® flow cytometer
- Cell suspension
- Medium appropriate for your cells
- Capped tubes or bottles for preparing reagent solutions, sterile preferred
- Serological pipets, sterile
- Micropipettor, single, 8 or 12 channel
- Disposable micropipettor tips, sterile preferred
- Vortex mixer
- Disposable gloves
- Guava® Instrument Cleaning Fluid (ICF) [Cat. No. 4200-0140]
- 100% Bleach solution containing between 5-6% hypochlorite
- Deionized water

**For High-Throughput (HT) Guava® Systems**

- 96-well microplates, round bottom (Falcon Cat. No. 353910 or 353918), flat bottom (Falcon Cat. No. 353075 or 353915), or hydrogel-coated flat bottom (Costar Cat. No. 3474, Myriad Industries Cat. No. 0400-3474), or equivalent. Refer to the appropriate Guava® System user's guide for compatible microplates.
- 0.5-mL microcentrifuge tubes for sample acquisition. Refer to the appropriate Guava® easyCyte™ System User's Guide for other compatible tubes.
- 1.5-mL microcentrifuge tubes for cleaning. Refer to the appropriate Guava® easyCyte™ System User's Guide for other compatible tubes.
- Reagent reservoirs, 50 mL (VWR Cat. No. 82026-354 or equivalent)
- V-shaped 25-mL and/or 55-mL troughs (25 mL, Apogent Discoveries Cat. No. 8093 and 55 mL, VWR Cat. No. 210070-970).

**For Single-Loader (SL) Guava® Systems**

- 1.5-mL microcentrifuge tubes, or 1.2-mL titer tubes for sample acquisition. Refer to the appropriate Guava® easyCyte™ System User's Guide for other compatible tubes.

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## Warnings and Precautions

1. For Research Use Only. Not for use in diagnostic procedures.
2. Wear appropriate personal protective equipment (PPE), including a lab coat and disposable gloves, when performing procedures. Wash your hands thoroughly after performing the test.
3. The Guava ViaCount Flex Reagent contains 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 SDS for specific information on hazardous materials.
4. The Guava ViaCount Flex Reagent 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.
5. Anhydrous DMSO is toxic. Exercise standard precautions when obtaining, handling, and disposing of toxic chemicals.
6. Avoid microbial contamination of the solution, which may cause erroneous results.
7. Handle all samples as if infectious using safe laboratory procedures such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories*, and in the *CLSI Document M29 Protection of Laboratory Workers from Occupationally Acquired Infections*.
8. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous result.
9. The fluorescent dyes in this reagent are light sensitive. Store in the dark and shield from excessive exposure to light.

10. During storage and shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For maximum recovery of the product, centrifuge the vial briefly prior to removing the cap.
11. Do not pipette by mouth.
12. Perform the procedure given in this package insert as described. Any deviation from the outlined protocols may result in assay failure or cause erroneous results.
13. Do not use the kit or any kit components past the expiration date indicated on the kit carton label. Do not interchange kit components from different kit lots. Lot numbers are identified on the kit label.
14. Follow your institution's safety procedures for working with chemicals and handling biological samples.
15. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
16. Safety Data Sheets (SDS) for kit reagents are available by contacting Luminex Corporation or visiting our website at [www.luminexcorp.com](http://www.luminexcorp.com)

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## Reagent Storage, Handling, and Stability

- Store Guava® ViaCount™ Flex reagent frozen at -15 to -25°C for long-term storage. Refer to the expiration date on the package label. We do not recommend using the reagent past the expiration date.
- ViaCount Flex reagent contains light-sensitive dyes. Shield from excessive exposure to light.
- ViaCount Flex reagent may be refrozen at -15 to -25°C for storage. The reagent may be repeatedly thawed and refrozen up to 10 times.

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## Protocol

### Before You Begin

This protocol was developed to allow direct count and viability determinations of multiple cell types. The kit provides reliable staining and results with cell concentrations in the range of  $1 \times 10^4$  to  $1 \times 10^7$  cells/mL.

It is highly recommended that cell samples be run shortly after the sample preparation has been completed. While some cell types have been shown to yield stable results for up to 4 hours after staining with the ViaCount™ Flex reagent, the stability of individual cell types may vary.

Optimal staining times and fluorescent signals can vary from cell line to cell line with ViaCount Flex reagent. For example, stained Jurkat (human T-lymphocytic leukemia) cells exhibit higher mean fluorescence intensities than HL60 (human promyelocytic leukemia) cells when incubated with ViaCount Flex reagent for 5 minutes. Some cell lines exhibit incompatibility when incubated with ViaCount Flex for extended periods of time, therefore you may need to determine optimal post-preparation times for your cell line. Although counting and viability results are not usually affected by this variation, optimal fluorescent signals can make analysis easier and more consistent.

**Time considerations:** The cell staining procedure with Guava® ViaCount Flex reagent takes 5 minutes. Acquiring data on your Guava System takes less than 2 minutes per sample. However, preparing cells for testing may require periodic maintenance and cultivation several days in advance. Always run a Guava Check or easyCheck™ prior to performing the assay. For details refer to the appropriate Guava System user's guide.

## Assay Considerations

The concentration and number of the cell samples for analysis will dictate the appropriate choice of reagent and sample preparation methods. If your cell sample is at a low to moderate density ( $1 \times 10^4$  to  $5 \times 10^5$  cells/mL), Guava<sup>®</sup> ViaCount<sup>™</sup> Flex reagent should be added directly to the cell suspension. If your cell sample is from a cell line that is incompatible with the standard Guava ViaCount reagent (eg, SF-9 cells), and is at a moderate to high density ( $1 \times 10^5$  to  $1 \times 10^7$  cells/mL or higher), the cell sample must be diluted with culture medium or a compatible buffer to a concentration of  $1 \times 10^4$  to  $5 \times 10^5$  cells/mL. See *Cell Sample Preparation* on page 29 for preparing cellular samples. See the tables in Staining Protocol for recommended sample dilution preparation volumes per test. If you have a large number of these types of samples to assay at one time, it will be more convenient to prepare a custom diluted Guava ViaCount Flex reagent formulation using compatible buffer or culture medium, and perform the assay as described in *Prepare and Stain Samples* on page 4.

This protocol and the automated HT Guava Systems were optimized to use round-bottom microplates (Falcon Cat. No. 3531190 or 353918, or equivalent). Flat bottom microplates (Falcon Cat. No. 353075 or 353915, or equivalent) have also been used to generate accurate results but will have a larger dead volume of approximately 75  $\mu$ L (versus <50  $\mu$ L in round-bottom plates). Refer to the appropriate Guava System user's guide for information on compatible microplates.

## Dilute the Reagent

**IMPORTANT NOTE:** Thaw the Guava<sup>®</sup> ViaCount<sup>™</sup> Flex reagent completely and mix well before use. Prepare only enough diluted Guava ViaCount Flex for immediate use to avoid problems with microbial contamination. The reagent may not be sterile. Do not freeze the diluted reagent formulation. Discard any unused diluted reagent.

If the expected cell concentration is between  $1 \times 10^4$  and  $5 \times 10^5$  cells/mL use Guava ViaCount Flex reagent undiluted.

1. Add the appropriate amount of Guava ViaCount Flex to an appropriate sized tube as described in Table 1.
2. Add the appropriate amount of culture medium or buffer as described in Table 1.
3. Mix well until the reagent is fully dissolved. There should be no dark purple reagent visible at the bottom of the tube, and the solution should appear pink.

**Table 1:** Dilution of ViaCount<sup>™</sup> Flex Reagent with Media

No. of Samples	ViaCount <sup>™</sup> Flex Reagent	Medium/Buffer
1	2 $\mu$ L	0.188 mL
10	20 $\mu$ L	1.88 mL
100	200 $\mu$ L	18.8 mL
* Some cell lines need a higher concentration of ViaCount <sup>™</sup> Flex reagent. In those cases add additional reagent to the medium/buffer.		

## Prepare and Stain Samples

1. Mix the cellular sample well by vortexing or repeated pipetting.
2. Transfer the appropriate volume of the cell suspension to a tube/well, as described in Table 2 and Table 3.

3. Add the appropriate volume of the Guava® ViaCount™ Flex (either diluted or stock, as determined above) to the sample well/tube. Mix well until the solution appears homogeneously pink in color. There should be no dark purple reagent visible at the bottom of the tube.
4. Incubate the sample for a minimum of 5 minutes at room temperature to allow dye staining to equilibrate.
5. The sample is ready for data acquisition on the Guava System. Mix the cell sample well before loading it onto the instrument.

**Table 2:** Low Concentration Samples ( $1 \times 10^4$  to  $5 \times 10^5$  cells/mL)

Concentration of Cell Suspension (cells/mL)	Cell Dilution Factor	ViaCount™ Flex Reagent	Cell Volume
$1 \times 10^4$ to $5 \times 10^5$	1	1 $\mu$ L	200 $\mu$ L

**Table 3:** Moderate to High Concentration Samples

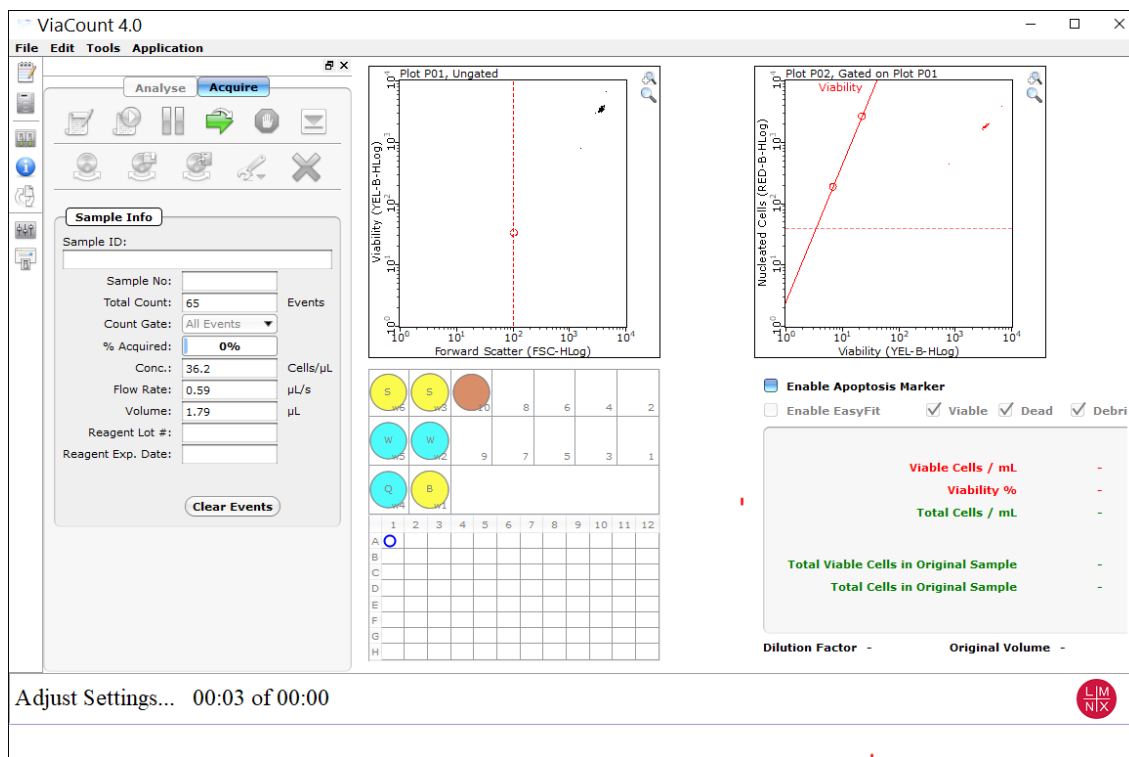
Concentration of Cell Suspension (cells/mL)	Cell Dilution Factor	ViaCount™ Flex Reagent	Cell Volume
$5 \times 10^5$ to $1 \times 10^6$	10	180 $\mu$ L	20 $\mu$ L
$1 \times 10^6$ to $1 \times 10^7$	20	190 $\mu$ L	10 $\mu$ L
$1 \times 10^7$ to $2 \times 10^7$	40	195 $\mu$ L	5 $\mu$ L
* Further dilution will be required for samples at cell concentrations greater than $2 \times 10^7$ .			

## Run the Assay on a High-throughput HT System

For details on software screen buttons and control panels, see the appropriate Guava® easyCyte™ User's Guide.

1. Prepare samples for analysis in a microplate or sample tubes.
2. Open GuavaSoft™ Software and click **ViaCount** from the Favorites list. If you do not see the assay listed in the Favorites list, click the **Program Search** drop-down menu, choose the assay, and click **Launch**.

**NOTE:** If the tray is ejected, click **OK** in the dialog box to load the tray. Be sure to keep the area clear as the tray loads.



3. Create a worklist file for the run before getting started. See the appropriate Guava® easyCyte™ User's Guide for details.
4. Click the **Edit Worklist** button, the first button in the Button panel, or select **Application > Worklist Editor** to open Worklist Editor and define the worklist parameters.

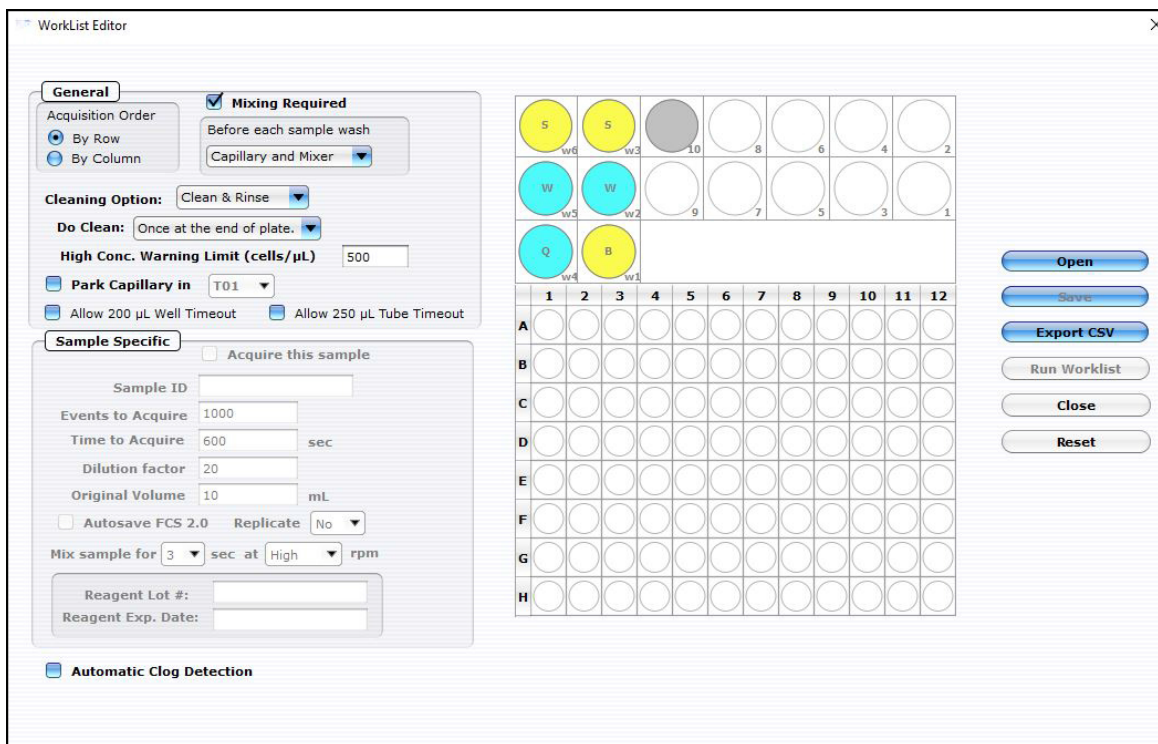
**NOTE:** You can also open an existing worklist.

- a. To open an existing worklist, click the **Start Worklist** button from the Button panel. The **Open WorkList File** dialog box displays.
- b. Choose between opening a saved worklist or running the currently loaded worklist. Worklists are .xml files.

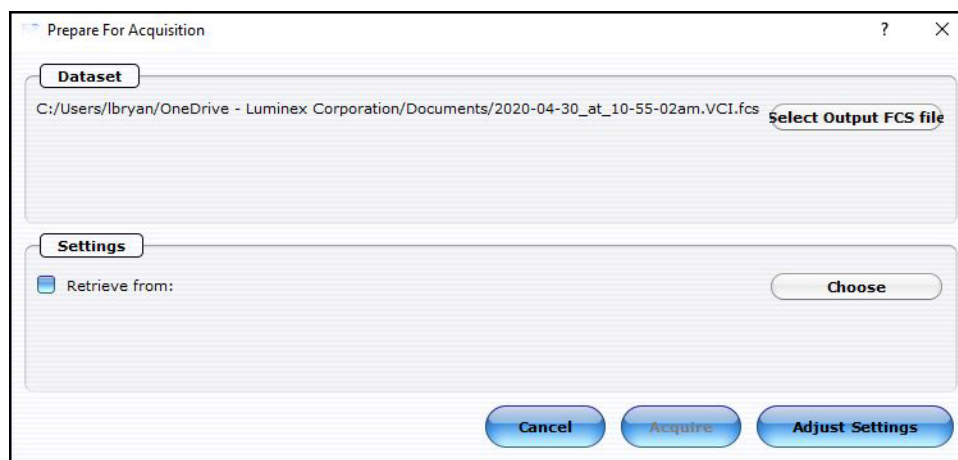


- c. Or, from the WorkList Editor, click **Open**.

- d. Choose the WorkList file and click **Run Worklist**. The tray ejects and a dialog box displays prompting you to enter a data set file name.



5. Define the worklist parameters in WorkList Editor software and click **Run Worklist**. When you start a worklist, the sample tray ejects. The **Prepare For Acquisition** dialog box displays allowing you to select a data file name and location and instrument settings.



- a. Click **Select Output FCS file** to save the file. The **Create New Session File** dialog box displays. Navigate to the location you want to save the file to, and click **Save**.

**NOTE:** The default storage location is in My Documents. The default file name is the date and time.

- b. For **Settings**, click **Choose**. The Read Settings from dialog box displays. Navigate to the .gst or .fcs file you want to open, and click **Open**.

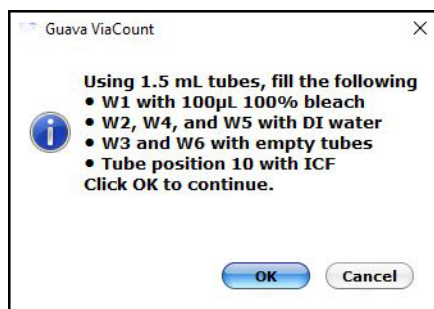


**NOTE:** The FCS or instrument settings file must be from InCyte, version 3.0, or later.

**NOTE:** Always save GuavaSoft data files directly to the system's hard drive during acquisition. Saving data to a network or location other than the computer's hard drive may result in data loss. You may copy the files to another location when acquisition is complete.

- Click **Adjust Settings**. If you retrieved instrument settings and you want to skip the adjust settings step, click **Acquire** to begin acquiring the first sample.

**NOTE:** Whether you are ready to acquire or adjust settings, a dialog box displays prompting you to load your samples.

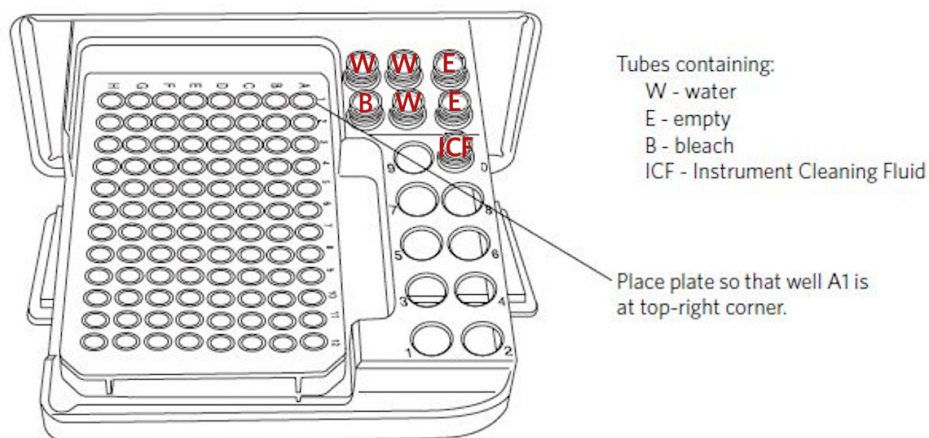


- Place the microplate or sample tubes, as well as the cleaning tubes in the tray. Make sure well A1 of the plate is in the top-right corner. Load the following 1.5-mL microcentrifuge tubes in these positions:


- Load tubes containing 1.5 mL of water in positions w2, w4, and w5 (for Quick Clean and washing the capillary and mixer).

**NOTE:** Ensure that the tube in position w4 (for Quick Clean) is filled with water.

- Load empty tubes in positions w3 and w6 (for spinning/drying the mixer).
- Load a tube containing 100 µL of 100% bleach in position w1 (for a backflush) to disinfect material deposited from a backflush.
- Load a tube containing 1.5 mL of ICF in position 10 for Clean & Rinse.
- Load a tube for parking the capillary.



- Click **OK** in the dialog box after you are finished loading samples and cleaning tubes to load the sample tray.

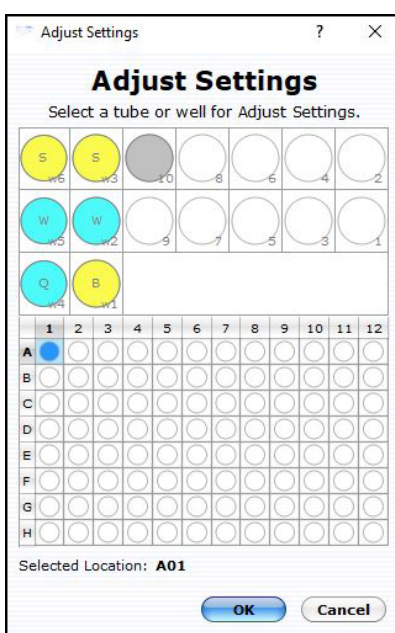


Keep the area clear as the tray loads.

Always use the **Eject Tray** button in the InCyte software module to open the door. Click **Pause** first, if necessary. Never open the door with your fingers.

## Adjust the Instrument Settings and Acquire Data

- A dialog box displays prompting you to select the sample for adjusting settings. Click to select the well or tube (1-9) used to adjust settings, then click **OK**. Luminex recommends using a stained negative or positive control sample for the initial adjustments. You can choose to skip the adjust settings step even if you did not retrieve settings, however Luminex recommends always performing the adjust settings step.



- Check the **Conc** (Cells/  $\mu$ L) value in the Sample Info pane and ensure that it is less than or equal to 500.

**NOTE:** If the value is greater than the high limit for the corresponding flow rate, dilute the sample with the appropriate buffer to lower the concentration and minimize the risk of coincident events. For optimal performance, Luminex recommends a concentration of 250 cells/ $\mu$ L or lower.

3. To fine tune the settings, you can make the following adjustments using the Gain. Open each instrument adjustment window using the Tools menu or the icons in the tool bar (left edge of the application window). If necessary, click the window's title bar to drag the window to a new location.

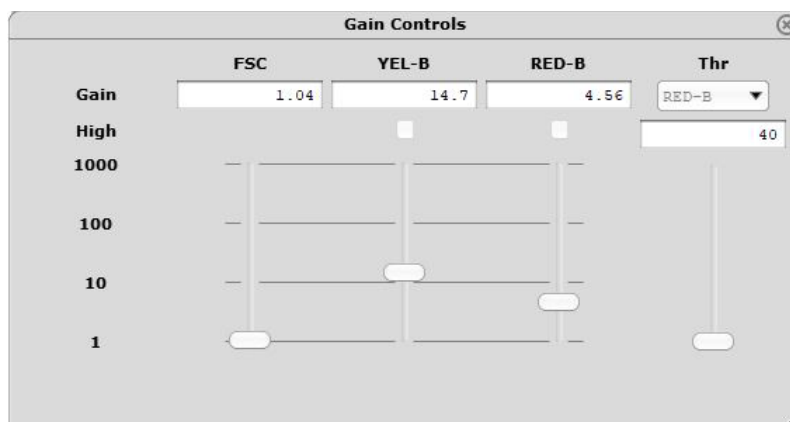


- a. Click the **Gain Controls** icon. The **Gain Controls** dialog box will display. Fine tune the settings only if necessary. You can make the following adjustments once events start to appear on the screen:
  - Set the **Refresh Rate** to the maximum number of events you want to display.
  - Use the **FSC Gain** setting to reduce or amplify the FSC signal so that the cells are visible and on scale. For optimal EasyFit Analysis, position the live cells at 10E3.
  - Adjust the gains (using the YEL-B and RED-B sliders or the arrow keys on the keyboard) so that the viable cells are positioned in the upper left and the dead cells are in the upper right of the Viability (YEL-B) vs. Nucleated Cells (RED-B) plot. For optimal EasyFit Analysis, position the live cells between 10E0 and 10E1 on the Viability (YEL-B) axis and between 10E2 and 10E3 on the Nucleated Cells (RED-B) axis. All dead cells should be above 10E2 but below saturation (4000 on the RED-B axis).
  - Adjust the horizontal line (RED-B threshold) on the Viability (YEL-B) vs. Nucleated Cells (RED-B) dot plot to separate the viable cells in the upper left from the debris in the lower left. Be sure to exclude all debris.
  - Adjust the vertical line (viability discriminator) on the Viability (YEL-B) vs. Nucleated Cells (RED-B) dot plot to separate the viable cells in the upper left from the non-viable cells in the upper right. You can adjust the angle of the line.
  - To adjust the FSC threshold, click and drag the vertical marker up or down the FSC axis of the FSC vs. Viability (YEL-B) dot plot until the desired amount of debris is eliminated below the marker.

**NOTE:** It is important to properly adjust the FSC threshold even if you plan to analyze using the EasyFit feature. Although the FSC threshold is not used during EasyFit Analysis, the FSC threshold is used as a counting gate to determine the total number of events acquired. If you set the thresh-

old too low, a large number of non-cellular events will be acquired rather than events of interest—live and dead cells.

**NOTE:** Use the **Clear Events** button in the Sample Info panel to clear the display.



4. When you are finished adjusting settings, click the **Next Step** button.
  - If necessary, you can repeat the adjust settings step to ensure that other samples (such as another positive control) are on scale, appropriately positioned, and compensated, by clicking **Adjust Settings**, loading the sample, and clicking **OK**.
  - If you want to save the instrument settings, click the **Save Settings** icon in the control panel. Enter a file name and click **Save** to save a .gst file.

**NOTE:** Once the worklist is complete, you can no longer save the instrument settings.

5. Click the **Resume Worklist** button. The system acquires the first sample.

**NOTE:** You may click the **Pause Worklist** button at any time during the run to select **Eject Tray** or **Capillary Cleaning Tools** then select **Backflush**, **Clean & Rinse**, or **Quick Clean**. The system will complete the current step before pausing. Click **Resume Worklist** to continue.

**NOTE:** If you want to adjust the instrument settings during the run, click **Pause Worklist**, then **Adjust Settings**. When the settings are set, click **Next Step**, then **Resume Worklist**.

**NOTE:** The % Acquired progress bar provides an estimate of the target event count during the acquisition period.

**NOTE:** The plate map in the control panel provides a visual status of acquisition. The well currently being acquired appears with an open blue circle. Wells acquired appear as a solid blue circle.

The system automatically performs a Quick Clean or a Clean & Rinse at the end of the assay. At the completion of the worklist, a copy of the file is automatically loaded into the Analysis control panel. Guava ViaCount saves the data for all samples as a single FCS 3.0 file to the specified location.

The FCS file contains:

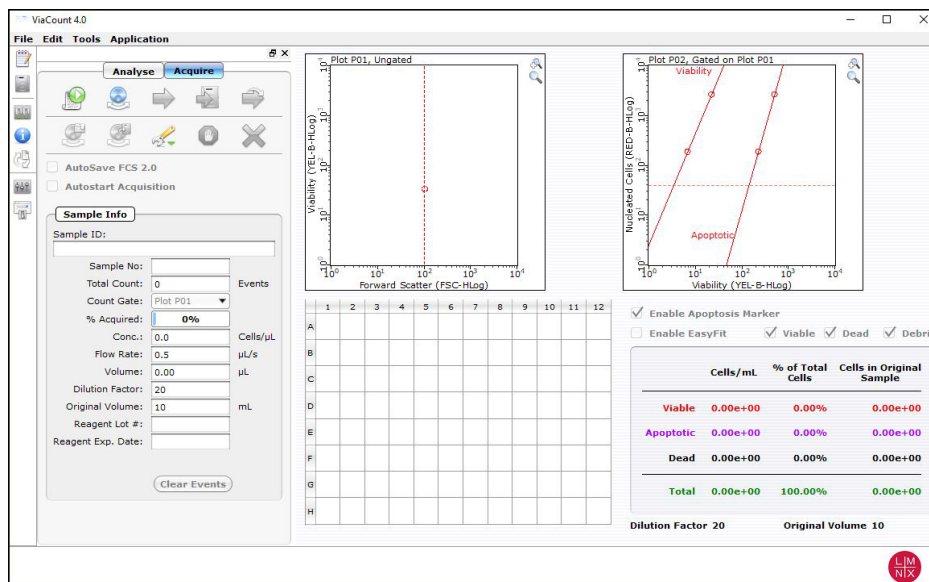
- the acquired data for all tubes in the run
- instrument settings (gains, compensation, miscellaneous settings)

6. If necessary, back up data files, to free up hard disc space.

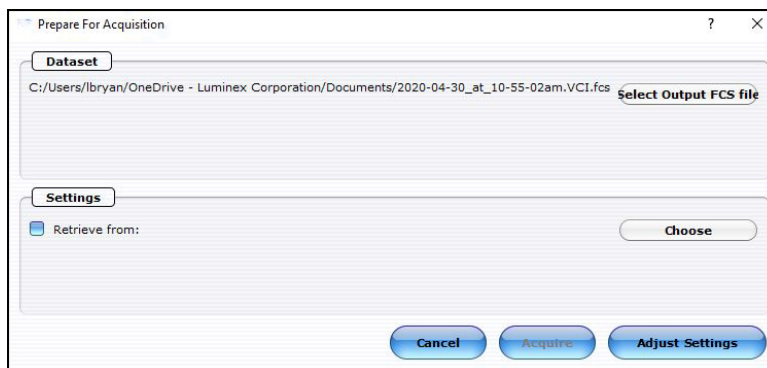
## Run the Assay on a Single-loader (SL) System

**NOTE:** The Guava® ViaCount™ screen opens in Acquisition mode, if the easyCyte™ System is turned on.

1. Open GuavaSoft™ Software and click **ViaCount** from the Favorites list. If you do not see the assay listed in the Favorites list, click the **Program Search** drop-down menu, choose the assay, and click **Launch**.



2. Prepare samples for acquisition in sample tubes.
3. Click the **Start New Session File** icon. A **Prepare For Acquisition** dialog box displays allowing you to choose a data file name and location and instrument settings.



- a. Click **Select Output FCS** file to save the file. The **Create New Session File** dialog box displays. Navigate to the location you want to save the file to, and click **Save**.

**NOTE:** The default storage location is in My Documents. The default file name is the date and time.

- b. For **Settings**, click **Choose**. The **Read Settings** from dialog box displays. Navigate to the .gst or .fcs file you want to open, and click **Open**.

**NOTE:** The FCS or instrument settings file must be from InCyte, version 3.0, or later.

**NOTE:** Always save GuavaSoft Software data files directly to the laptop's hard drive during acquisition. Saving data files to a network or location other than the laptop's hard drive may result in data loss. You may copy the file(s) to another location when acquisition is complete.

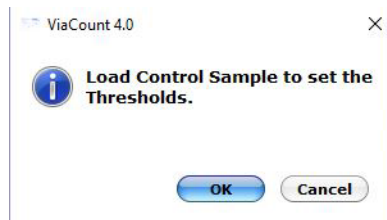
**NOTE:** Your system administrator may have configured GuavaSoft Software to disable the retrieval of Methods, instrument settings, and compensation settings from Method files and individual instrument settings files. If the Settings and Compensation retrieval options are disabled, you will not be

able to adjust instrument settings. However, you can retrieve settings and Methods from a single FCS file.

4. Click **Adjust Settings**.

**NOTE:** If you have already retrieved instrument settings and you want to skip the Adjust Settings step, click **Acquire** in the **Prepare for Acquisition** dialog box to proceed to acquisition. A dialog box displays prompting you to load the control sample into the instrument.

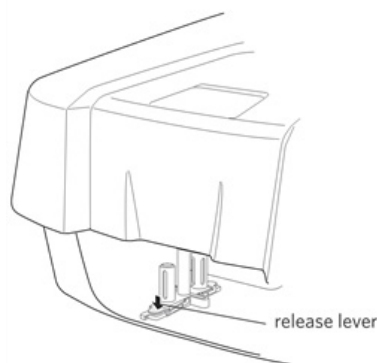
5. Mix a stained cell sample and load the sample tube onto the sample loader of the Guava easyCyte System. Luminex recommends using a stained negative sample for the initial adjustments.
6. Click **OK** in the ViaCount 4.0 dialog box.



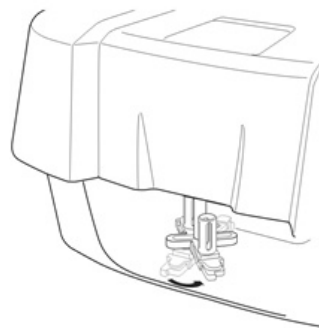
7. Adjust the appropriate settings for your experiment and once you are satisfied and ready to acquire data, click **Next Step**.

## Adjust the Instrument Settings

1. Mix a stained cell sample and load the sample tube on the sample loader of the Guava® easyCyte™ System. Luminex recommends using a stained negative or isotype control sample for the initial adjustments.
2. From the Guava ViaCount Acquisition screen, click the **Adjust Setting** icon from the Button panel.
  - a. To load sample tubes, pinch the release lever with your thumb and forefinger and gently lower the arm assembly (1st image).
  - b. Once lowered, rotate the assembly to change the sample tube (2nd image), then push up on the assembly until you hear a click.
  - c. If prompted, click **OK** in the **Messages** dialog box.



1. Pinch release lever and gently lower sample holder.



2. Rotate loader assembly to change tubes.

3. Check the **Conc** (Cells/ $\mu$ L) value in the **Sample Info** pane and ensure that it is less than or equal to 500.

**NOTE:** If the value is greater than the high limit for the corresponding flow rate, dilute the sample with the appropriate buffer to lower the concentration and minimize the risk of coincident events. For optimal performance, Luminex recommends a concentration of 250 cells/ $\mu$ L or lower.

4. To fine tune the settings, you can make the following adjustments using the Gain Controls. Open each instrument adjustment window using the Tools menu or the icons in the tool bar (left edge of the application window). If necessary, click the window's title bar to drag the window to a new location.

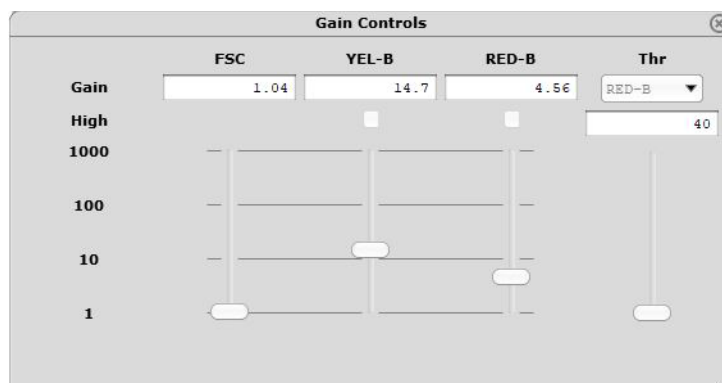


- a. Click the **Gain Controls** icon. The **Gain Controls** dialog box will display. Fine tune the settings only if necessary. You can make the following adjustments once events start to appear on the screen:
  - Set the **Refresh Rate** to the maximum number of events you want to display.
  - Use the **FSC Gain** setting to reduce or amplify the FSC signal so that the cells are visible and on scale. For optimal EasyFit Analysis, position the live cells at 10E3.
  - Adjust the gains (using the YEL-B and RED-B sliders or the arrow keys on the keyboard) so that the viable cells are positioned in the upper left and the dead cells are in the upper right of the Viability (YEL-B) vs. Nucleated Cells (RED-B) plot. For optimal EasyFit Analysis, position the live cells between 10E0 and 10E1 on the Viability (YEL-B) axis and between 10E2 and 10E3 on the Nucleated Cells (RED-B) axis. All dead cells should be above 10E2 but below saturation (4000 on the RED-B axis).
  - Adjust the horizontal line (RED-B threshold) on the Viability (YEL-B) vs. Nucleated Cells (RED-B) dot plot to separate the viable cells in the upper left from the debris in the lower left. Be sure to exclude all debris.
  - Adjust the vertical line (viability discriminator) on the Viability (YEL-B) vs. Nucleated Cells (RED-B) dot plot to separate the viable cells in the upper left from the non-viable cells in the upper right. You can adjust the angle of the line.
  - To adjust the FSC threshold, click and drag the vertical marker up or down the FSC axis of the FSC vs. Viability (YEL-B) dot plot until the desired amount of debris is eliminated below the marker.

**NOTE:** It is important to properly adjust the FSC threshold even if you plan to analyze using the EasyFit feature. Although the FSC threshold is not used during EasyFit Analysis, the FSC threshold is used as a counting gate to determine the total number of events acquired. If you set the thresh-

old too low, a large number of non-cellular events will be acquired rather than events of interest—live and dead cells.

**NOTE:** Use the **Clear Events** button in the Sample Info panel to clear the display.



5. When you are finished adjusting settings, click the **Next Step** button.
  - If necessary, you can repeat the adjust settings step to ensure that other samples (such as another positive control) are on scale, appropriately positioned, and compensated, by clicking **Adjust Settings**, loading the sample, and clicking **OK**.
  - If you want to save the instrument settings, click the **Save Settings** icon in the control panel. Enter a file name and click **Save** to save a .gst file.

**NOTE:** Once the worklist is complete, you can no longer save the instrument settings.

6. Vortex the first sample and load it on the instrument.
7. Enter the number of events to acquire in the Sample Info panel. The default number of events to acquire is 1000.
8. If you want to identify individual samples or sets of samples, enter an optional ID in the Sample ID field. The sample ID may be any text up to 40 characters long. If you do not enter a sample ID, the default sample number will be used as the sample ID, for example, Sample #1, Sample #2, etc.

**NOTE:** If you want to enter additional sample characteristics, you can do so with the Change Custom Field Labels feature and create up to 2 user defined labels. These fields are editable similar to the standard Sample ID. The Change Custom Field Labels feature is located in the Application menu.

**NOTE:** If you select **Autostart Acquisition**, acquisition automatically starts when you load each tube. You do not need to click **Acquire Next Sample**. Autostart Acquisition automatically turns off if you click any of the following: **Adjust Settings**, **Retrieve Settings**, **Quick Clean**, or **Backflush**. You must recheck the box to continue using the feature. If you click **Autostart Acquisition** and want to enter a sample ID, type the ID into the Sample ID field after acquisition is complete and before loading the next sample.

9. Click the **Acquire Next Sample** button. The system acquires the first sample.

**NOTE:** The progress bar provides an estimate of the target event count during the acquisition period.

**NOTE:** If a clog or total loss of signal is detected, we recommend removing the clog by running a Quick Clean with water, then a Quick Clean with ICF, followed by Backflush into an empty tube, then another Quick Clean with ICF, and a final Quick Clean with water.

**NOTE:** If the acquisition rate appears to slow dramatically, the fluid pathway may be blocked. Click the **Abort** button, then click **Capillary Cleaning Tools** and select **Backflush**. Load a tube of 20% bleach



and click **OK**. When the backflush is complete, select **Quick Clean**. Load a tube of DI water and click **OK**. Click **Acquire Next Sample** to continue.

10. Click **Save and Close Current Sample**. You may still enter or change the Sample ID for the current sample before clicking **Save and Close Current Sample**.
11. Repeat steps for the remaining samples.
12. Click the **Stop and Close Session File** after the last sample.
13. When you are finished, click the **Capillary Cleaning Tools** icon in the Button panel and select **Quick Clean**. Load a tube of deionized water and click **OK**.

**NOTE:** If you ran blood samples, run two Quick Clean cycles with water, followed by one cycle using ICF, and finally one cycle using water to rinse.

At the completion of the run, a copy of the Data, Method, and AnalysedGroup are automatically loaded into the Analysis control panel. Guava ViaCount saves the data for all samples as a single FCS 3.0 file to the specified location.

The FCS file contains:

- the acquired data for all tubes in the run
- instrument settings (gains, compensation, miscellaneous settings)

## EasyFit Analysis

You may select Enable EasyFit and allow the software to calculate the results based on its own internal analysis method. Using a 3-dimensional software algorithm, EasyFit groups the events into three populations—viable cells, dead/apoptotic cells, and debris.

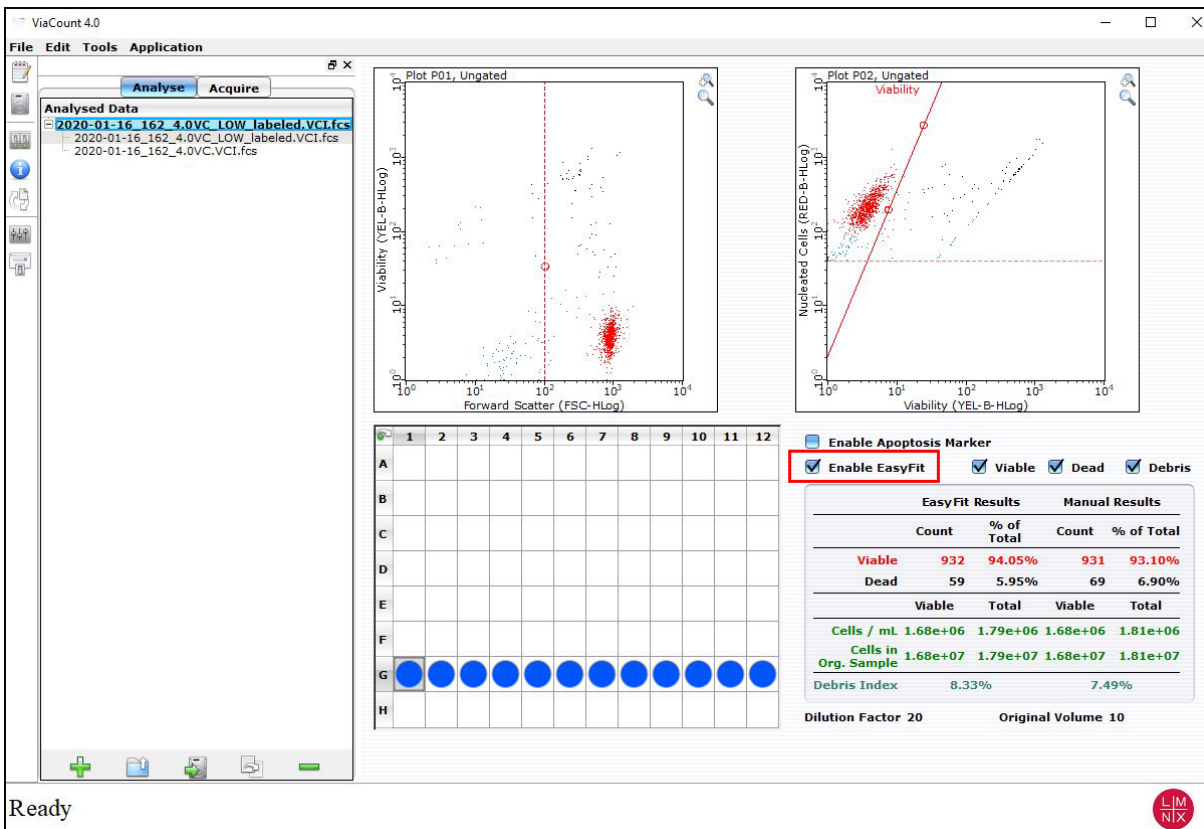
You may switch between EasyFit and manual analysis to see which method may be more appropriate for the given data. When EasyFit Analysis is selected, the FSC threshold and viability marker appear in gray for reference, but they cannot be adjusted. EasyFit Analysis uses its own method of grouping (clustering) data into populations. The threshold and viability marker remain on the plots so that you can compare the EasyFit results with the manual results, which are also displayed. When the EasyFit feature is disabled, you may, if necessary, adjust the FSC threshold and viability marker to appropriately discriminate debris from cells, as well as live cells from dead/apoptotic cells.

**NOTE:** The apoptosis gate is not available for EasyFit Analysis. Apoptotic cells are included in the EasyFit dead cell population. If you want to calculate individual results for dead and apoptotic cells, use the manual analysis method and set an apoptosis gate.

To hide or show the events within specific populations, use the check boxes to the left of the Viability (YEL-B) vs. Nucleated Cell (RED-B) dot plot. The selection you make applies to the data while you are viewing it. The selections are not saved to the file.

**NOTE:** Before routinely using EasyFit for your analysis, you should validate the EasyFit Analysis method for the cells you are testing by running multiple experiments and comparing the EasyFit results to another cell counting and viability test method, such as manual ViaCount™ analysis. In some cases EasyFit Analysis will provide more accurate results because it uses 3-dimensional gating to identify live and dead/apop-

totoc cells. However, even after validating EasyFit Analysis, compare the EasyFit results to the results obtained with the manual method, and check the plots for each sample to ensure the analysis is correct.

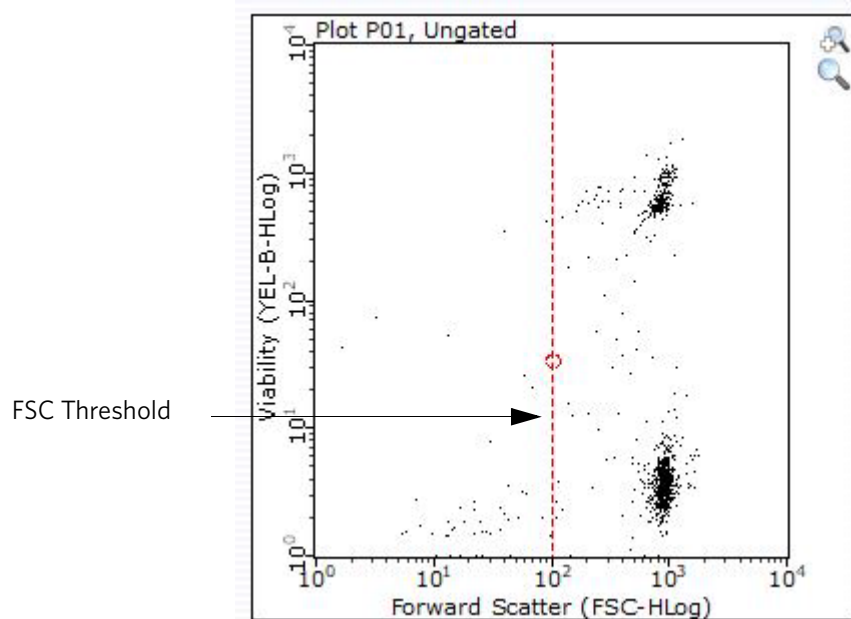


The manual results—results obtained from the FSC threshold and viability marker, which you left at their defaults or modified—appear next to the EasyFit results.

## Manual Analysis

1. To analyze manually, clear the **Enable EasyFit** check box.
2. If necessary, you may adjust the FSC threshold during analysis. During acquisition, the FSC threshold is used as a counting gate—any event that passes the threshold is included in the Events to Acquire. After acquisition is complete, if you move the threshold, the total cell count will change. However, by moving the threshold you

may be able to correct for debris that was included or cellular events that were not included during acquisition. You cannot adjust the FSC threshold if EasyFit is enabled.

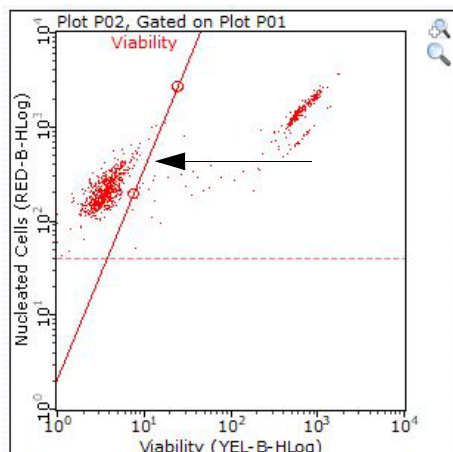


Adjust FSC threshold, if necessary, when analyzing manually.

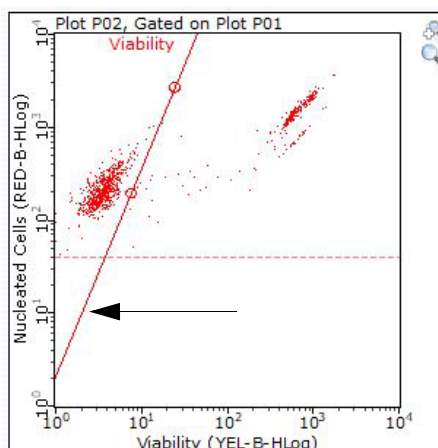
- If necessary, you may adjust the location and angle of the red viability marker to more precisely discriminate viable from non-viable cells (or to separate dead cells from live and apoptotic cells).

**NOTE:** If you are using EasyFit Analysis, viable cells versus dead and apoptotic cells are determined by the software. The viability marker will be ignored.

- To adjust the angle of the marker, click and drag the top of the line to tilt it to the desired location.
- To move the entire marker to the left or right, click and drag the bottom of the line to shift it to the desired location.



Click and drag top of line to adjust angle.

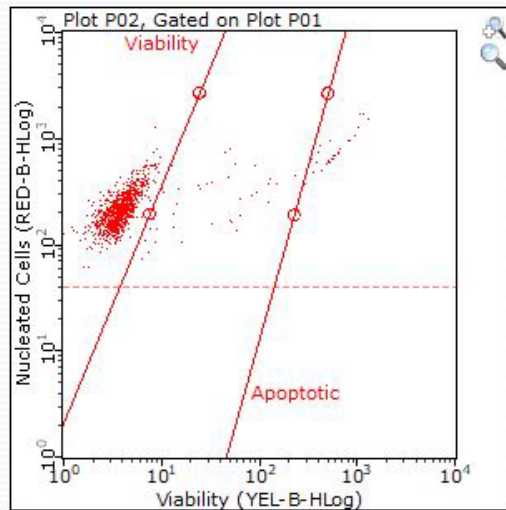


Click and drag bottom of line to move location.

Viability marker adjusted to separate viable cells (left) from apoptotic and dead cells (right).

4. To set the apoptosis marker, click **Enable Apoptosis Gate**. The apoptosis marker is available during manual analysis. It is not available when EasyFit is enabled.
5. To set the apoptosis marker, follow the same instructions outlined above for setting the Viability Marker.

**NOTE:** Make sure the apoptosis marker is to the right of the viability marker and that the markers are not intersecting.



**Enable Apoptosis Marker**

Click to enable Apoptosis marker.

Viability marker separating viable cells (left) from apoptotic cells (right). Apoptosis marker separating apoptotic cells (left) from dead cells (right).

## Guava® ViaCount™ Results

Guava® ViaCount™ results display immediately after the acquisition of the sample is complete. The results displayed vary depending on whether the EasyFit or manual method of analysis was used and if the apoptosis marker was set. Following are examples of results from each type of analysis.

**Figure 1: ViaCount™ manual results for viability marker**

<b>Viable Cells / mL</b>	<b>1.68e+06</b>
<b>Viability %</b>	<b>93.10%</b>
<b>Total Cells / mL</b>	<b>1.81e+06</b>
<b>Total Viable Cells in Original Sample</b>	<b>1.68e+07</b>
<b>Total Cells in Original Sample</b>	<b>1.81e+07</b>

Results include statistics for viable and total cells:

- Number of viable cells per mL
- Percentage of viable cells
- Total number cells per mL
- Number of viable cells in original sample
- Total number of cells in original sample

The dilution factor and original volume that you entered are displayed.

**Figure 2: ViaCount™ manual results for viability and apoptosis markers**

	<b>Cells/mL</b>	<b>% of Total Cells</b>	<b>Cells in Original Sample</b>
<b>Viable</b>	<b>1.68e+06</b>	<b>93.10%</b>	<b>1.68e+07</b>
<b>Apoptotic</b>	<b>6.51e+04</b>	<b>3.60%</b>	<b>6.51e+05</b>
<b>Dead</b>	<b>5.96e+04</b>	<b>3.30%</b>	<b>5.96e+05</b>
<b>Total</b>	<b>1.81e+06</b>	<b>100.00%</b>	<b>1.81e+07</b>

Results include statistics for viable, apoptotic, dead, and total cells:

- Number of cells per mL
- Percentage of cells
- Number of cells in original sample

The dilution factor and original volume that you entered are displayed.

**Figure 3: ViaCount™ EasyFit results (manual results also included)**

	EasyFit Results		Manual Results	
	Count	% of Total	Count	% of Total
<b>Viable</b>	<b>932</b>	<b>94.05%</b>	<b>931</b>	<b>93.10%</b>
<b>Dead</b>	<b>59</b>	<b>5.95%</b>	<b>69</b>	<b>6.90%</b>
	<b>Viable</b>	<b>Total</b>	<b>Viable</b>	<b>Total</b>
<b>Cells / mL</b>	<b>1.68e+06</b>	<b>1.79e+06</b>	<b>1.68e+06</b>	<b>1.81e+06</b>
<b>Cells in Org. Sample</b>	<b>1.68e+07</b>	<b>1.79e+07</b>	<b>1.68e+07</b>	<b>1.81e+07</b>
<b>Debris Index</b>	<b>8.33%</b>		<b>7.49%</b>	

EasyFit results appear along side the manual results. Results include:

- Number of viable and dead cells in sample (count)
- Percentage of viable and dead cells in sample (count)
- Number of viable cells per mL
- Total number of cells per mL
- Number of viable cells in original sample
- Total number of cells in original sample
- Debris index (The debris index is a measure of debris. The higher the index the more debris in the sample.)

$$\frac{\text{\# of debris events}}{\text{\# of total cells} + \text{\# of debris events}} \times 100$$

## Guava® Excel Utility

The Guava® Excel Utility Program can be used to extract pertinent analysis information (cell concentration, percent viability, dilution factor, and original volume) from the CSV file and create an Excel spreadsheet file. The information is organized in the following four worksheets of the Excel workbook:

- manual analysis summary sheet
- EasyFit Analysis summary sheet (If EasyFit was not enabled, "--" appears in the fields under the EasyFit columns.)
- manual analysis sheet (a subset of relevant information found in the CSV)
- data sheet (contains the same information found in the CSV file)

The utility program can be used by all GuavaSoft™ software users including administrators, supervisors, and operators. You may use the program to open CSV files created in CytoSoft Software, version 2.0 and later.

**NOTE:** The utility cannot be used if the apoptosis gate was enabled.

To open a file with the utility program, you can either use the drag-and-drop method or start the program.

**NOTE:** If a dialog box appears indicating that the document contains macros, select the **Enable Macros** option.

## Drag and Drop CSV File

1. Locate GuavaExcelUtil.exe icon. It is located in the C:\Program Files\Millipore\guavaSoft\GuavaExcelUtil directory along with a Templates folder that the program uses.
2. Drag and drop a CSV file onto the utility icon. The utility program starts Microsoft Excel and creates a workbook with the appropriate information. The Excel file is saved automatically. If you make changes to the file, you must save the changes. The file name assigned to the Excel file is the same name as the CSV file but with the extension .xls. The original CSV file is unchanged.
3. Excel automatically write protects the summary sheet. To disable the protection, select **Protection** from the Tools menu in the Excel menu bar. Then choose **Unprotect Sheet** from the list of options.

**NOTE:** If you want to place a copy of the Guava® Excel Utility Program on the desktop, you must also place a copy of the Templates folder on the desktop with the executable.

## Open the Utility Program

1. Locate GuavaExcelUtil.exe icon (or shortcut) and double-click to start the program.
2. Choose a CSV file from the file dialog box. The utility program starts Microsoft Excel and creates a workbook with the appropriate information. The file name assigned to the Excel file is the same name as the CSV file but with the extension .xls. The original CSV file is unchanged.
3. Excel automatically write protects the summary sheet. To disable the protection, select **Protection** from the Tools menu in the Excel menu bar. Then choose **Unprotect Sheet** from the list of options.

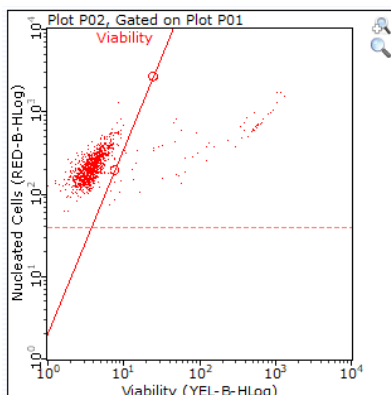
## Expected Results

ViaCount™ Flex reagent allows for accurate and precise determinations of cell concentration and viability of cultures in microplates. The ViaCount Software Module in GuavaSoft™ Software performs calculations automatically, with results displayed on the computer screen after each sample is acquired. Results are automatically exported to a spreadsheet file, which can be analyzed using a program such as Microsoft Excel®. Refer to the appropriate Guava® System user's guide for more information about the ViaCount Assay.

Using ViaCount Flex reagent, samples at concentrations as low as  $1 \times 10^4$  cells/mL can be assayed directly for cell count and viability. Hemacytometer counts cannot be done accurately for samples at this low a concentration without manipulating the cell sample first. Concentrating the sample can introduce additional error in your counts. With ViaCount Flex reagent, only 200  $\mu$ L of the cell suspension is needed (2000 cells from a sample at a concentration of  $1 \times 10^4$  cells/mL) for an accurate and precise cell count and viability assessment. For example, for a microplate containing a low concentration of Jurkat cell samples in ViaCount Flex reagent, the average total cell concentration across the plate was of  $1.3 \times 10^4$  cells/mL with a 6.8% CV, and the average viability was 82% with a 2.7% CV. In general, for all cell concentrations and percent viabilities tested, the accuracy of the ViaCount Flex Assay is +/-10% with a precision of <10% for cell concentration and an accuracy of +/-5% with a precision of <5% for percent viability.

ViaCount Flex reagent also allows you to assay other challenging samples with added flexibility in your protocol design. Non-mammalian cell lines can be assayed easily over an extended time period for your convenience. With this concentrated reagent, you can customize the formulation to optimize assay performance for difficult cell lines. For cell samples in poorer condition, containing more apoptotic and dying cells, the ViaCount Flex reagent pro-

vides a tight pattern that allows better discrimination between the viable and non-viable population, making gating easier and more consistent.



<b>Viable Cells / mL</b>	<b>1.68e+06</b>
<b>Viability %</b>	<b>93.10%</b>
<b>Total Cells / mL</b>	<b>1.81e+06</b>

## Troubleshooting Tips

- Careful, accurate pipetting with calibrated pipettors is required for accurate and consistent results in the ViaCount™ Assay. If you are using multichannel pipettors, ensure that all tips are firmly seated. Mix cell samples well. Cells will settle to the bottom of the tube or well over short periods of time.
- Thaw ViaCount Flex reagent formulations completely and mix well before using.
- To avoid bacterial contamination problems, prepare only enough ViaCount Flex reagent diluted in medium for immediate use. This reagent cannot be sterilized by filtration, because the dyes in the ViaCount Flex reagent bind to the filter membrane. Do not autoclave the ViaCount Flex reagent. We do not recommend adding preservatives without testing for cell sensitivity or toxicity.
- We recommend keeping the concentration of DMSO in a test sample at 1% or below when using ViaCount Flex reagent. If you follow the dilutions recommended in this protocol, the concentration of DMSO will be 1% in the well when the final concentration of ViaCount Flex reagent is 1:100.
- Ensure that the mixing option has been selected in the Worklist file used to collect data in the ViaCount Software Module. Cells begin to settle immediately and cell concentrations and percent viabilities will be inaccurate unless the Guava system mixes each sample just prior to acquisition.
- Even though we have shown a number of cell types (Jurkat, HL60, CHO) to be stable in ViaCount Flex reagent for up to 6 hours, we recommend acquiring data from test samples as soon as possible after staining incubation is complete, due to the varying nature of different cell lines. Until you have demonstrated that your particular cells are stable over time in ViaCount Flex reagent, maintain stained cell samples under conditions favorable to the cells until data acquisition. Leaving samples at room temperature for long periods of time or other suboptimal handling conditions before data acquisition can lead to low viability.
- Use Guava® ICF at least once per day to clear the fluid system of buildup from cell samples and reagent residue. Run Guava Clean with Guava ICF followed by water after finishing ViaCount Assays. To disinfect as well as clean the Guava System, mix 1 part bleach with 9 parts Guava ICF and use that mixture in place of the Guava ICF only.
- Cell handling can affect cell staining with the ViaCount Flex reagent. When removing adherent cells from the culture substrate using enzymatic treatment (for example, ViaCount Cell Dispersal reagent or Trypsin), take care not to damage the cells by over-digestion. If the culture yields unexpectedly low viability counts, allow



the cells to recover in culture medium for 20 to 30 minutes before assaying a fresh aliquot of the cell suspension with ViaCount Flex reagent.

9. Run Guava Check (Cat. No. 4500-0020) or easyCheck™ (Cat. No. 4500-0025) to verify proper instrument function and accuracy.
10. 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 either insufficient volume to continue to acquire sample, or a blockage of the flow system. Check first for the lack of sufficient sample volume by pausing GuavaSoft™ Software and ejecting the tray. If the sample volume is below 50 µL, there is not enough sample for the instrument to acquire. Either add additional buffer to bring the sample volume up to greater than 50 µL, or proceed to the next sample. If the sample volume is more than 50 µL, then the lack of events acquired is probably due to a clog. A clog or blockage of the flow system can be caused by cell aggregates, cell debris, bleach crystals, or other particulates. Click **Backflush** to flush out the clog into a tube containing 20% bleach. Then run a Quick Clean cycle to remove bleach residue. If this procedure does not alleviate the problem, refer to the appropriate Guava System user's guide or contact Technical Support for additional help.

For more troubleshooting tips, refer to the appropriate Guava System user's guide.

# Guava® ViaCount™ Assay Troubleshooting

Problem	Possible Cause	Solutions
Message: <i>This file already exists. You must pick a new name.</i>	Spreadsheet file with same file name already exists in selected directory.	Save ViaCount™ spreadsheet file to another directory or give it a new name.
Message: <i>This file exists with read-only attributes. Please use a different file name.</i>	FCS file with same file name already exists in selected directory.	Save ViaCount FCS file to another directory or give it a new name.
Guava ViaCount Software Module starts in Analysis mode. Acquisition mode is not available.	<ol style="list-style-type: none"> <li>1. Communication problem between instrument and laptop.</li> <li>2. A registration code was not entered or was entered incorrectly.</li> </ol>	<ol style="list-style-type: none"> <li>1. Ensure USB cable is connected between instrument and laptop.</li> <li>2. Enter the registration code. The code is case sensitive.</li> </ol>
Message: <i>Less than 10 particles/μL. Sample is too dilute. Accuracy may be compromised.</i>	<ol style="list-style-type: none"> <li>1. Sample concentration is too low.</li> <li>2. Clogged flow cell.</li> <li>3. Settings were not adjusted correctly.</li> </ol>	<ol style="list-style-type: none"> <li>1. Recommended concentration range for accurate counting is <math>1 \times 10^4</math> to <math>5 \times 10^5</math> cells/mL.</li> <li>2. Perform a Backflush. Follow with Quick Clean.</li> <li>3. Adjust Settings and make necessary changes.</li> </ol>
Message: <i>More than 500 particles/μL. Sample is too concentrated. Please dilute or accuracy may be compromised.</i>	Sample is too concentrated.	Recommended concentration range for accurate counting is $1 \times 10^4$ to $5 \times 10^5$ cells/mL.

Problem	Possible Cause	Solutions
Few events, as indicated in Particle Count section of Sample Information control panel.	<ol style="list-style-type: none"> <li>1. Clogged flow cell.</li> <li>2. Insufficient sample volume.</li> <li>3. Cells in suspension have settled.</li> <li>4. Cells were lysed during staining.</li> <li>5. Sample was not properly stained.</li> </ol>	<ol style="list-style-type: none"> <li>1. Perform a Backflush. Follow with Quick Clean.</li> <li>2. Minimum sample volume is 100 µL for round-bottom wells, 150 µL for 0.5-mL tubes, and 900 µL for 1.5-mL tubes for HT systems and 90 µL for single-loader systems.</li> <li>3. Ensure sample mixing option was selected in WorkEdit Software.</li> <li>4. Check sample. If necessary, restain sample from original suspension.</li> <li>5. Check buffers used to process cells. Check expiration date of reagent.</li> </ol>
No events, as indicated in Particle Count section of Sample Information control panel.	<ol style="list-style-type: none"> <li>1. Sample tube or plate not loaded.</li> <li>2. Insufficient sample volume.</li> <li>3. Clogged flow cell.</li> <li>4. Broken flow cell.</li> <li>5. Sample pump not working.</li> <li>6. Laser not operational.</li> <li>7. Loose fitting on minstac tubing (under metal plate).</li> </ol>	<ol style="list-style-type: none"> <li>1. Ensure tube or plate is in place and tray is loaded.</li> <li>2. Minimum sample volume is 100 µL for round-bottom wells, 150 µL for 0.5-mL tubes, and 900 µL for 1.5-mL tubes for HT systems, and 90 µL for single-loader systems.</li> <li>3. Perform a Backflush. Follow with Quick Clean.</li> <li>4. Remove flow cell and inspect for damage. Replace if necessary.</li> <li>5. Run Quick Clean and watch for fluid in waste vial.</li> <li>6. Contact Luminex Technical Support.</li> <li>7. Ensure tubing connector is secure.</li> </ol>
Events appear off scale in dot plots.	FSC gain or PM1 and /or PM2 voltages set incorrectly, or samples staining brightly.	Adjust gain setting or voltage settings so positive populations appear on scale.

Problem	Possible Cause	Solutions
Viability is significantly lower than expected.	<ol style="list-style-type: none"><li data-bbox="610 268 993 338">1. Sample was not mixed properly before acquisition.</li><li data-bbox="610 443 993 470">2. Residual bleach in flow cell.</li><li data-bbox="610 512 993 611">3. Buffer used is non-isotonic or pH is out of physiological range.</li><li data-bbox="610 617 993 686">4. Cells are fragile and stressed by preparation procedure.</li><li data-bbox="610 716 993 814">5. ViaCount Reagent may be incompatible with non-mammalian cell line.</li></ol>	<ol style="list-style-type: none"><li data-bbox="1006 268 1377 436">1. Ensure sample mixing option was selected in WorkEdit Software, or click Abort, remove sample, mix, and reload.</li><li data-bbox="1006 443 1377 512">2. Run Quick Clean. Restain fresh cell sample.</li><li data-bbox="1006 512 1377 581">3. Check osmolarity and pH. pH should be 7.2-7.4.</li><li data-bbox="1006 617 1377 751">4. Resuspend cells in medium containing 10-20% FBS for 30 min with frequent, gentle agitation. Restain.</li><li data-bbox="1006 751 1377 779">5. Use ViaCount Flex Reagent.</li></ol>

Problem	Possible Cause	Solutions
<p>Cell count is significantly different than expected.</p>	<ol style="list-style-type: none"> <li>1. Dilution factor or original volume entered incorrectly.</li> <li>2. Sample was not mixed properly before acquisition.</li> <li>3. Adherent cells were not adequately removed from culture surface.</li> <li>4. Adherent cells adhere to wells in plate</li> <li>5. Sample not stained sufficiently</li> <li>6. Sample was not stained completely.</li> <li>7. Sample dilution factor is too low.</li> <li>8. Clogged flow cell.</li> <li>9. Cells are aggregated.</li> <li>10. Instrument settings not optimal.</li> <li>11. Incorrect sample tubes or plates used.</li> </ol>	<ol style="list-style-type: none"> <li>1. Ensure dilution factor and original volume are correct.</li> <li>2. Ensure sample mixing option was selected in WorkEdit Software, or click Abort, remove sample, mix, and reload.</li> <li>3. Check culture vessel to ensure cells were removed. Repeat procedure to release cells if necessary.</li> <li>4. Use the low binding plate for adherent cells</li> <li>5. Some heterogeneous cell populations such as whole blood or PBMCs may need up to 10 minutes for staining. Optimal timing may need to be determined.</li> <li>6. Ensure reagent is not expired or was not exposed to excessive light. Ensure sufficient amount of ViaCount Flex Reagent was used.</li> <li>7. Use at least a 1:10 of sample to Viacount Reagent, or use Viacount Flex CS Reagent (Catalog No. 4500-0110).</li> <li>8. Perform a Backflush. Follow with Quick Clean.</li> <li>9. Prepare uniform suspension. Use ViaCount CDR to disaggregate cells.</li> <li>10. Adjust settings so positive populations appear on scale and debris is below threshold.</li> <li>11. See Order Information for list of supported tubes and plates.</li> </ol>
<p>Guava Excel Utility will not start Microsoft Excel when you try to open a CSV file</p>	<ol style="list-style-type: none"> <li>1. File is not a ViaCount file or it was created by a version of CytoSoft Software prior to version 2.0.</li> <li>2. Templates folder is not located in same directory as utility program.</li> </ol>	<ol style="list-style-type: none"> <li>1. Excel Utility can be used only for ViaCount files created in CytoSoft Software, version 2.0 or later.</li> <li>2. Be sure Templates folder and executable are in same folder.</li> </ol>

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# Cell Sample Preparation

## Prepare Non-Adherent and Adherent Cells

The following protocols describe how to harvest non-adherent or adherent cells cultured in 96-well plates, as well as non-adherent or adherent cells cultured in flasks or other tissue culture vessels. Each of the culturing conditions requires different protocols to harvest the cells.

### Prepare Non-Adherent Cells

1. Set up initial culture conditions, such that after culture and treatment, cells are at a concentration of  $1 \times 10^5$  to  $1 \times 10^7$  cells/mL in serum- or albumin containing medium.
2. Proceed to *Prepare and Stain Samples* on page 4.

### Prepare 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, additional reagents such as Guava® Cell Dispersal Reagent (Catalog No. 4700-0050) may be used to dissociate clumps.

1. Using your preferred method for dissociation, detach the cells from their culture vessel.
2. Add fresh serum- or albumin-containing medium to each well so final concentration is between  $1 \times 10^5$  to  $1 \times 10^7$  cells/mL.
3. Proceed to *Prepare and Stain Samples* on page 4.

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## Limitations

1. The results of the assay are dependent upon proper use of reagents, products, and instruments.
2. The ViaCount™ Assay might not assess the Percent Viability and Total Cell Concentration of low viability cell suspensions as accurately as for higher viability samples. In general, the actual viability of cell suspensions with less than 20% live cells might be lower than reported by the ViaCount Flex reagent. This can be due to changes in cell structure and integrity affecting the staining properties of dead and dying cells. Excessive debris might also interfere with accurate counts. Some low results can be somewhat avoided by using optimal instrument and analysis settings. Call Luminex Technical Support for more information.
3. The Guava® System and ViaCount Flex reagent yield optimal results when the stained cell sample for acquisition is between  $1 \times 10^4$  to  $5 \times 10^5$  cells/mL. To obtain the most accurate counting results, adjust the concentrations to within the recommended range.
4. Cell samples containing cells of a size range between approximately 7 to 60 micrometers in diameter yield the most accurate results on the Guava System. If your cell samples contain significant amounts of cells outside this size range, call Luminex Technical Support for more information.
5. Cell samples containing aggregates might not yield accurate cell counts or percent viability results. Call Luminex Technical Support for more information on how to deal with aggregate samples.

6. The ViaCount Assay might not work with all cell lines. Certain cell types do not stain efficiently causing incorrect cell counts and/or viability results. Cell lines expressing fluorescent proteins (for example, transfectants expressing GFP, YFP, and so on) or other macro-molecules (for example, transfectants expressing non-fluorescent proteins) might yield accurate total cell counts but incorrect viable cell counts. The signal from the expressed transfected fluorescent protein or transfected product might be detected in one of the Guava Instrument's fluorescence channels (usually the one detecting stained dead cells). This can cause an inaccurately low viability reading for the culture.
7. Culturing conditions can affect cell staining with the ViaCount reagent. We have validated the ViaCount Assay on SF-9 and CHO-K1 cells grown in culture medium containing surfactants; however, unusual additives or medium components and drugs may interact with the reagent or affect the assay.
8. Using 96-well microplates that are incompatible with the Guava System can cause false or unexpected results. If you want to use plates other than those specified in *Materials Required but Not Provided* on page 1, refer to the appropriate Guava System user's guide for a complete list of plates that are compatible with the Guava System.

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