



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

**RUO**

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

4600-0010, Rev J

Catalog No. 4000-0040 (40 mL)

Catalog No. 4000-0041 (240 mL)

06/2020

## Technical Support

Telephone: 512-381-4397

North America Toll Free: 1-877-785-2323

International Toll Free: + 800-2939-4959

Email: [support@luminexcorp.com](mailto:support@luminexcorp.com)

[www.luminexcorp.com](http://www.luminexcorp.com)



**Luminex Corporation**

12212 Technology Blvd.

Austin, TX 78727

U.S.A.

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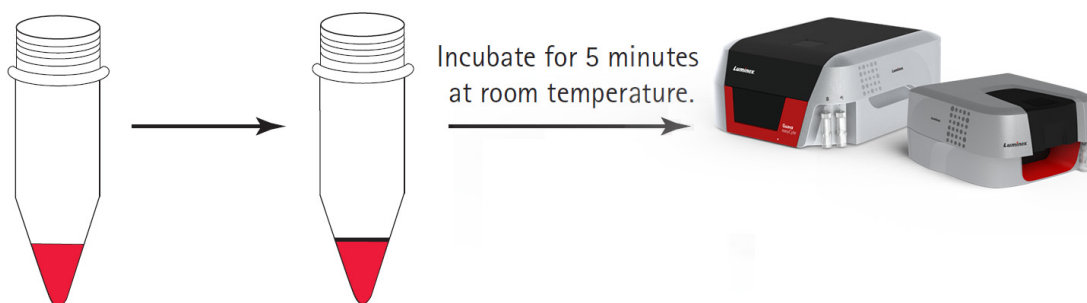
## Description

The Guava® ViaCount™ Reagent provides absolute cell count and viability data on cell suspensions from a variety of cultured mammalian cell lines. The Guava ViaCount Assay distinguishes between viable and non-viable cells based on the differential permeability of DNA-binding dyes in the Guava ViaCount Reagent. The fluorescence of each dye is resolved operationally to allow the quantitative assessment of both viable and non-viable cells present in a suspension. The Guava ViaCount Assay is intended as a rapid and reliable method for determining cell count and viability.

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## Summary of Protocol

Add ViaCount reagent\* to each tube.      Add cell suspension\* to each tube.



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## Materials Provided

- Guava® ViaCount™ Reagent (Cat. No. 11-25210, 40 mL; Cat. No. 11-25209, 240 mL)

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## Materials Required but Not Provided

- Guava® System with the Guava ViaCount™ Software Module
- Cell suspension
- Dilution buffer: Phosphate buffered saline (PBS), or equivalent balanced salt solution, pH 7.2 to 7.4. Buffer should not contain phenol red indicator.
- Micropipettors
- Disposable micropipettor tips
- Vortex mixer
- Disposable gloves

- 100% Bleach solution containing between 5-6% hypochlorite
- Deionized (DI) water

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

- 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.
- 96-well microplate plates, round bottom . Refer to the appropriate Guava® System user's guide for other compatible microplates.

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

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

## Additional User Supplied Materials

- Guava® easyCheck™ Kit (Cat. No. 4500-0025)
- Guava ViaCount™ Cell Dispersal Reagent for adherent cells (Cat. No. 4700-0050), optional
- Guava® Instrument Cleaning Fluid (ICF) (Cat. No. 4200-0140), optional

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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™ Reagent contains dyes that may be carcinogenic and/or mutagenic. Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic and mutagenic reagents.
4. The Guava ViaCount Reagent contains sodium azide, which is toxic. Contact with acids liberates toxic gas. Dispose of all materials according to federal, state or local regulations.
5. Avoid microbial contamination of the solution, which may cause erroneous results.
6. 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*.
7. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
8. Do not pipette by mouth.

9. 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.
10. 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.
11. Follow your institution's safety procedures for working with chemicals and handling biological samples.
12. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
13. 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)

## Reagent Storage, Handling, and Stability

- Store the Guava® ViaCount™ Reagent 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.
- The Guava ViaCount Reagent contains light-sensitive dyes. Shield from excessive exposure to light.

## Protocol

### Prepare and Stain Samples

1. Prepare a uniform cell suspension for counting. See *Cell Sample Preparation* on page 28. Be sure adherent cells are completely removed from flasks and are well mixed.
 

**NOTE:** Accurate cell counting requires the even distribution of the cells within suspensions. Gently but thoroughly mix all suspensions during all dilution and staining steps and before loading samples on the Guava® System for analysis. Do not vortex samples vigorously, as sample can splash out or cause cell damage resulting in erroneous cell counts.
2. Prepare stained cell samples by mixing cells with Guava ViaCount™ Reagent in a sample tube. Accurate cell counting on the Guava System occurs at a concentration range of  $1 \times 10^4$  up to  $5 \times 10^5$  cells/mL in the stained sample. If you know the approximate concentration of the original cell suspension, refer to the following table as a dilution guide.

**Table 1:** Cell Suspension Dilution Table (recommended volumes)

Conc. of Original Cell Suspension	Dilution Factor	Cell Suspension Volume	ViaCount Reagent Volume	Conc. of Diluted Cells
$1 \times 10^5$ to $1 \times 10^6$ cells/mL	10	50 $\mu$ L	450 $\mu$ L	$<1 \times 10^5$ cells/mL
$1 \times 10^6$ to $1 \times 10^7$ cells/mL	20	20 $\mu$ L	380 $\mu$ L	$<5 \times 10^5$ cells/mL
$>1 \times 10^7$ cells/mL	40*	20 $\mu$ L	780 $\mu$ L	$>2.5 \times 10^5$ cells/mL

Conc. of Original Cell Suspension	Dilution Factor	Cell Suspension Volume	ViaCount Reagent Volume	Conc. of Diluted Cells
* Further dilution may be necessary for highly concentrated cell suspensions.				

For highly concentrated cell suspensions ( $>1 \times 10^7$  cells/mL), we recommend that you start with a 40-fold dilution of your original cell suspension. You may need to further dilute the sample to attain a stained cell sample in the concentration range for accurate cell counting. For example, for cell suspensions, such as whole blood, of concentrations  $>2 \times 10^7$  cells/mL, you should prepare an 80-fold dilution of your original cell suspension.

If you do not know the approximate concentration of your original cell suspension, prepare a stained cell sample by mixing with ViaCount Reagent at a 20-fold dilution (for example, 20  $\mu\text{L}$  of cell suspension into 380  $\mu\text{L}$  of ViaCount Reagent). If your cell sample shows a Total Cells per mL value of  $1 \times 10^7$  or higher, we recommend that you prepare additional dilutions (for example, 40-fold and 80-fold dilutions) for the assay to verify the cell count. If the stained cell sample for data acquisition is too concentrated ( $>5 \times 10^5$  cells/mL), the cell count may not be accurate.

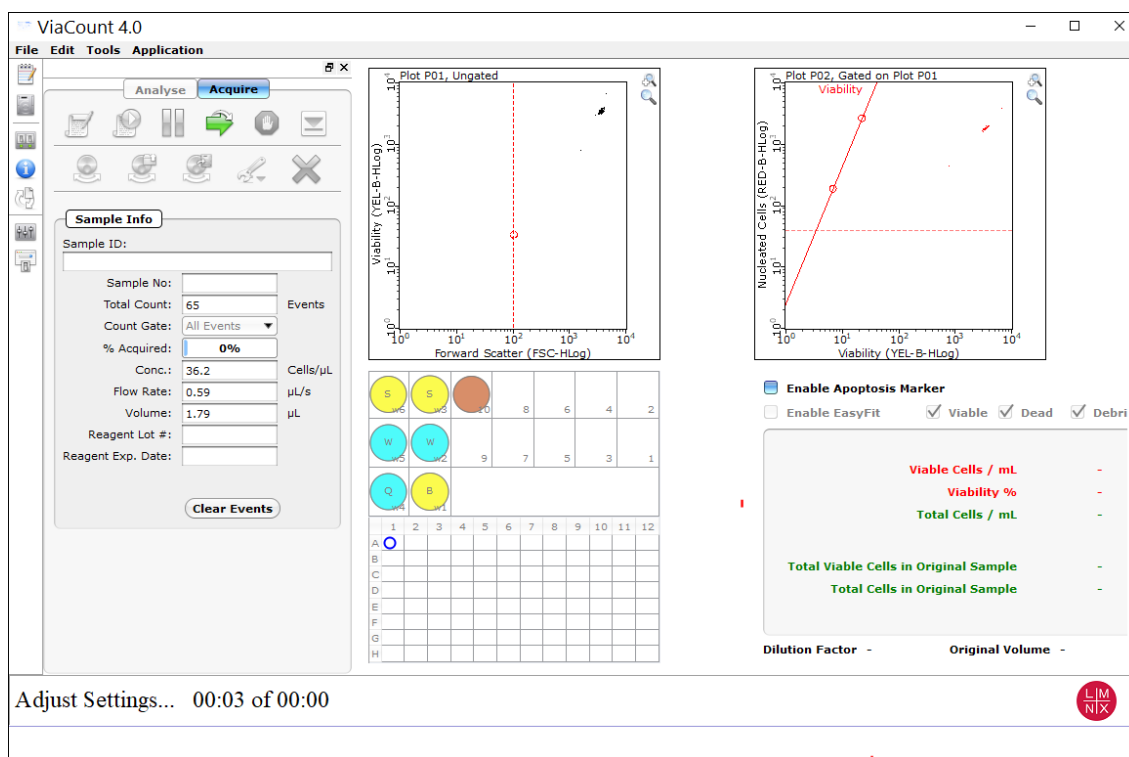
3. Allow the cells to stain for at least 5 minutes.

## 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**.

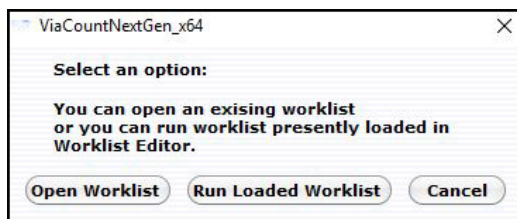
**NOTE:** If the tray is ejected, click **OK** in the dialog box to load the tray. Be sure to keep the area clear as the tray loads.



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.

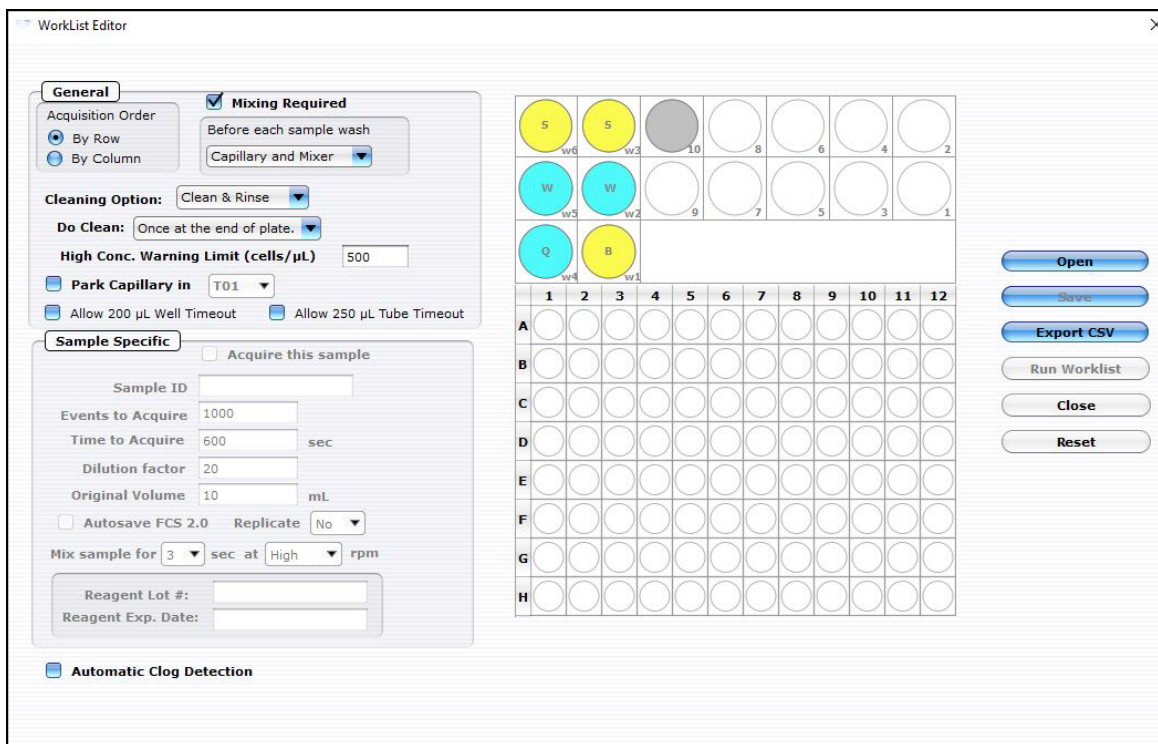
**NOTE:** You can also open an existing worklist.

- 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.

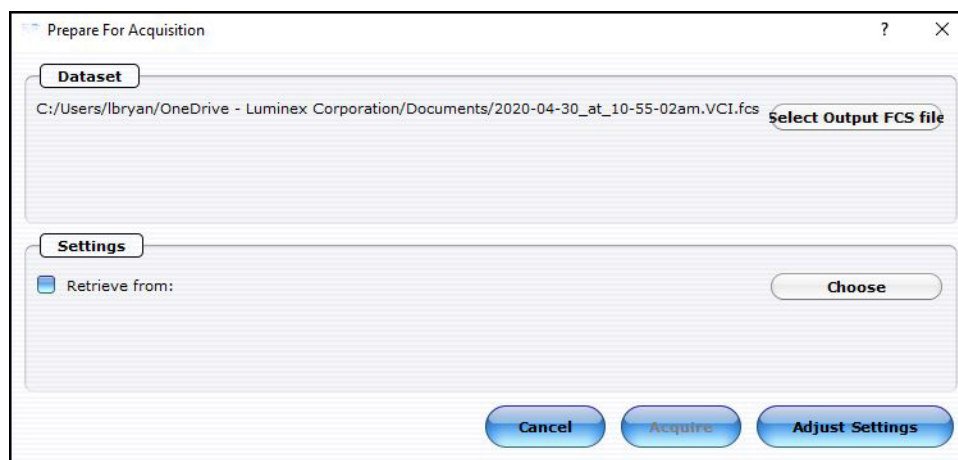


- c. Or, from the WorkList Editor, click **Open**.

- 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.

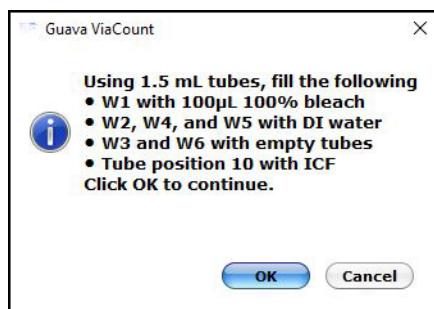
- b. For **Settings**, click **Choose**. The Read Settings from dialog box displays. Navigate to the .gst or .fcs file you want to open, and click **Open**.

**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.

6. 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.

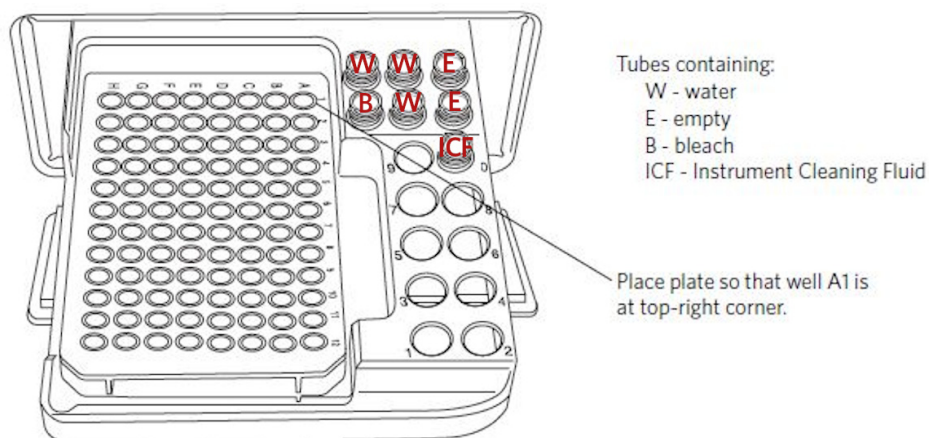


7. Place the microplate or sample tubes, as well as the cleaning tubes in the tray. Make sure well A1 of the plate is in the top-right corner. Load the following 1.5-mL microcentrifuge tubes in these positions:

- 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.

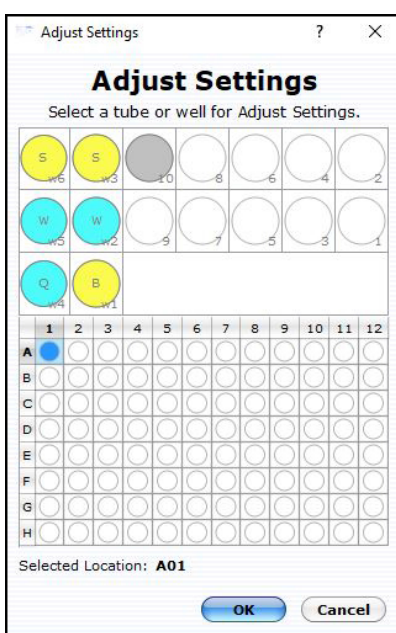


Keep the area clear as the tray loads.

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.

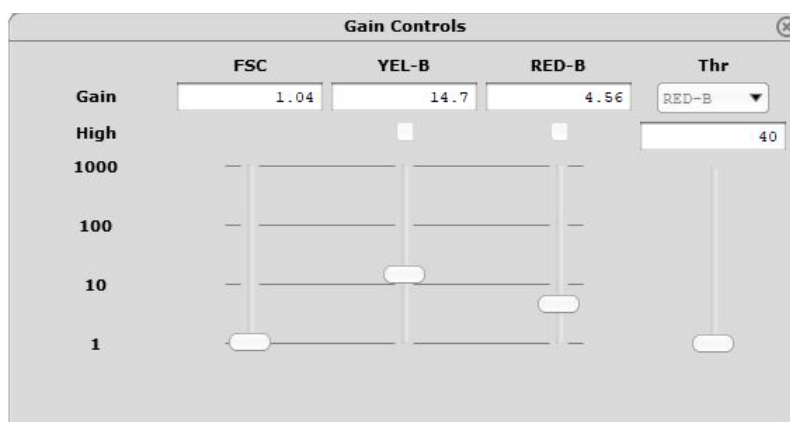


- a. Click the **Gain Controls** icon. The **Gain Controls** dialog box will display. Fine tune the settings only if necessary. You can make the following adjustments once events start to appear on the screen:
  - Set the **Refresh Rate** to the maximum number of events you want to display.
  - Use the **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:

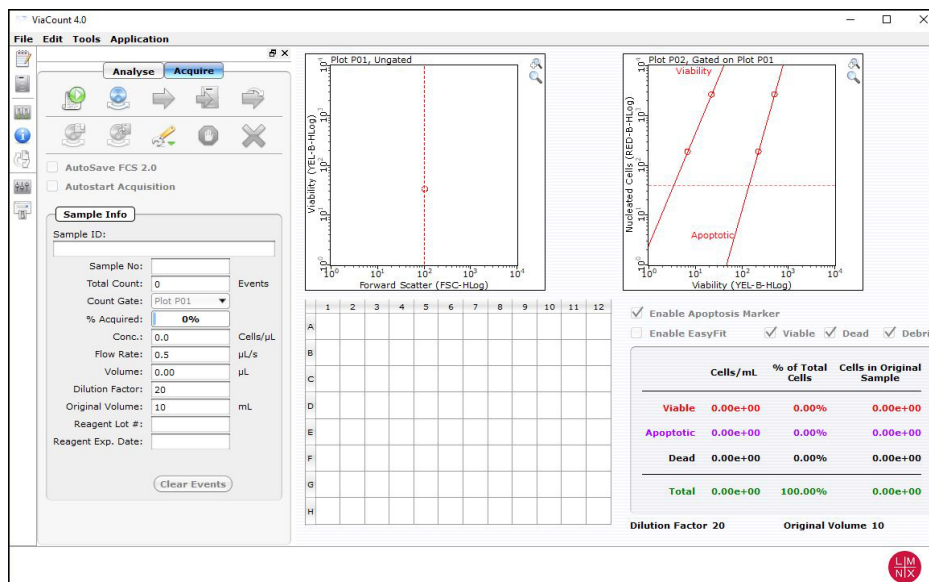
- the acquired data for all tubes in the run
- instrument settings (gains, compensation, miscellaneous settings)

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

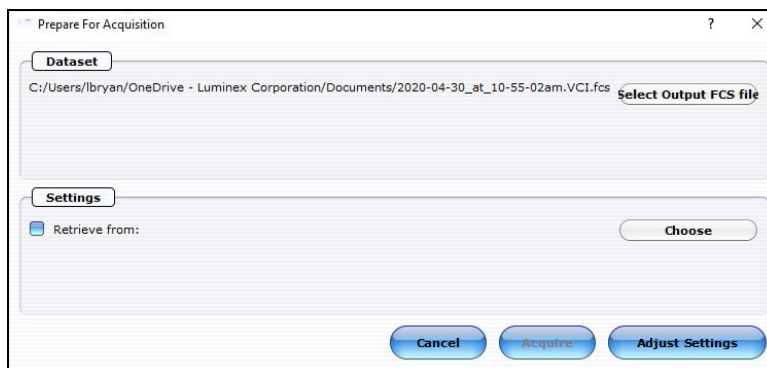
## Run the Assay on a Single-loader (SL) System

**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.

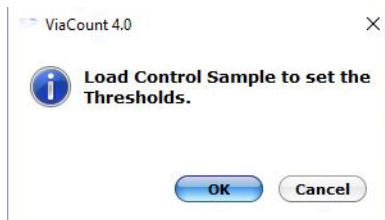
**NOTE:** Your system administrator may have configured GuavaSoft Software to disable the retrieval of Methods, instrument settings, and compensation settings from Method files and individual instrument settings files. If the Settings and Compensation retrieval options are disabled, you will not be

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.

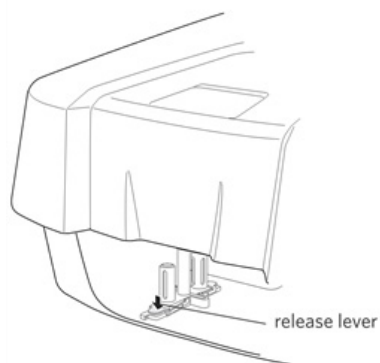
5. Mix a stained cell sample and load the sample tube onto the sample loader of the Guava easyCyte System. Luminex recommends using a stained negative sample for the initial adjustments.
6. Click **OK** in the ViaCount 4.0 dialog box.



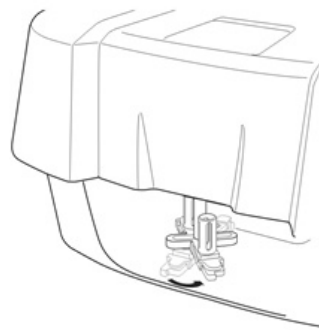
7. Adjust the appropriate settings for your experiment and once you are satisfied and ready to acquire data, click **Next Step**.

## 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.

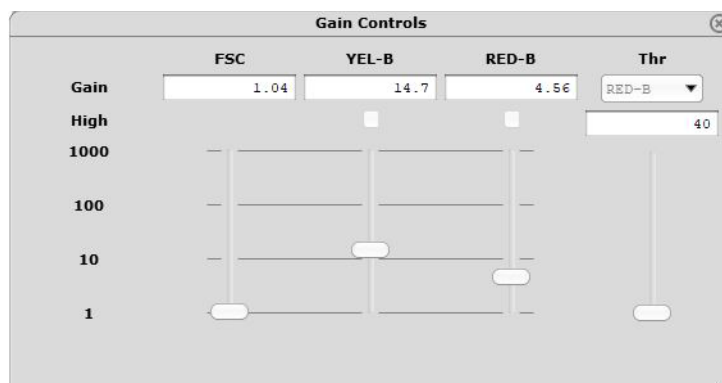


- a. Click the **Gain Controls** icon. The **Gain Controls** dialog box will display. Fine tune the settings only if necessary. You can make the following adjustments once events start to appear on the screen:
  - Set the **Refresh Rate** to the maximum number of events you want to display.
  - Use the **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.

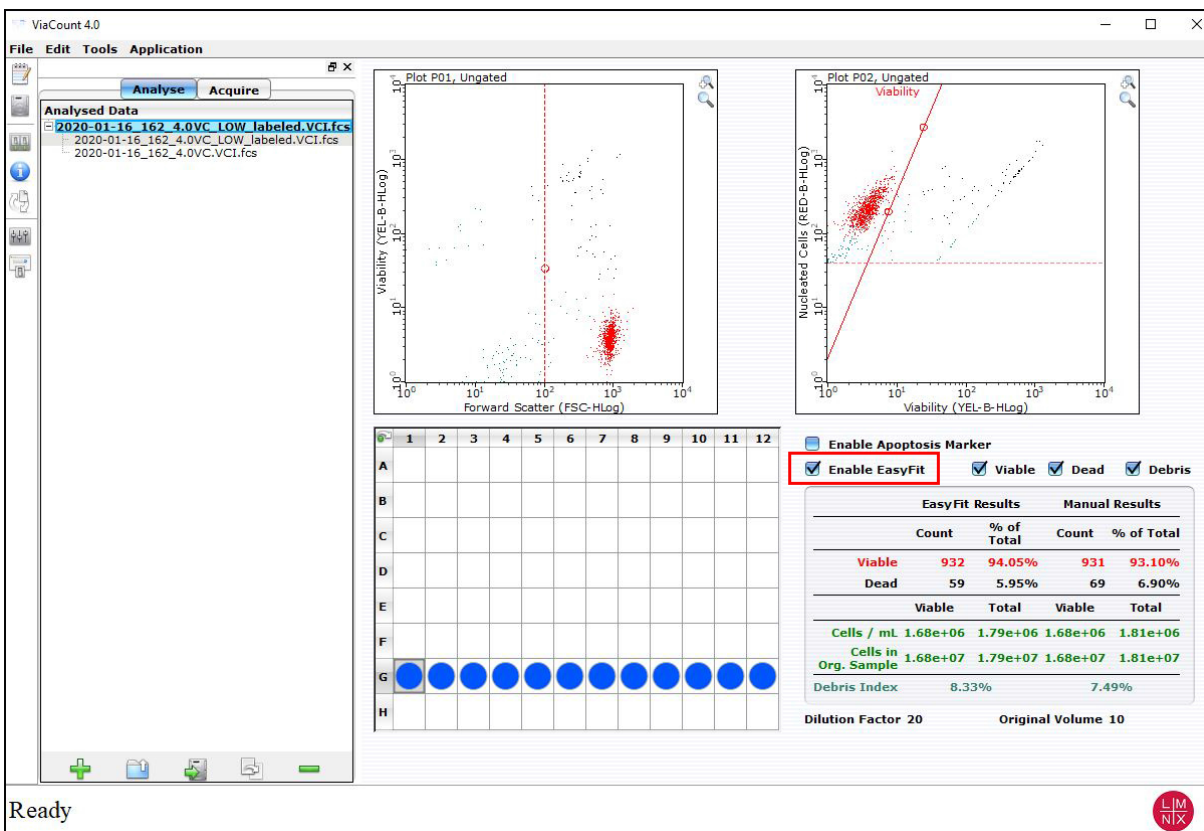
**NOTE:** The apoptosis gate is not available for EasyFit Analysis. Apoptotic cells are included in the EasyFit dead cell population. If you want to calculate individual results for dead and apoptotic cells, use the manual analysis method and set an apoptosis gate.

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.

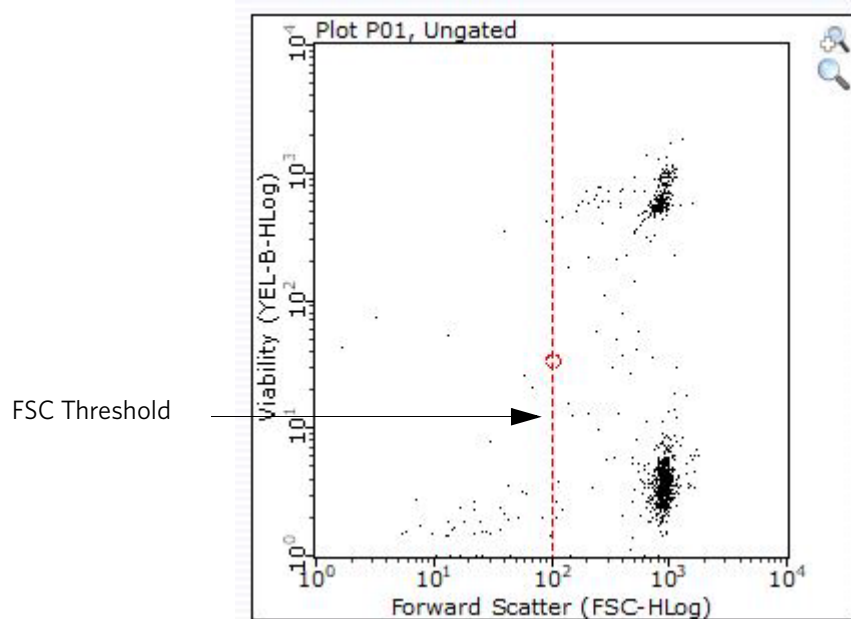


The manual results—results obtained from the FSC threshold and viability marker, which you left at their defaults or modified—appear next to the EasyFit results.

## 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.

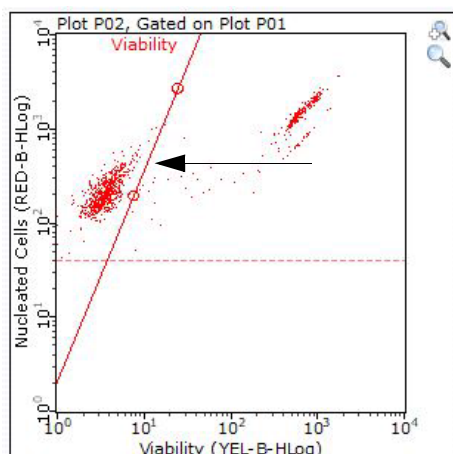


Adjust FSC threshold, if necessary, when analyzing manually.

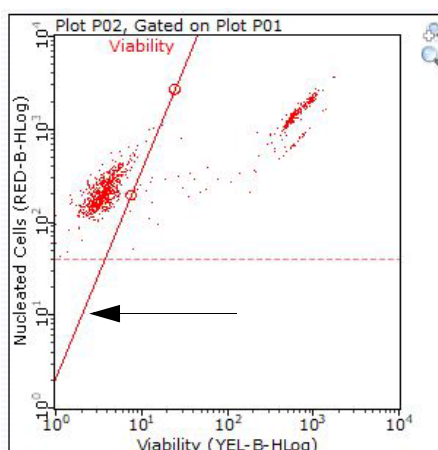
- If necessary, you may adjust the location and angle of the red viability marker to more precisely discriminate viable from non-viable cells (or to separate dead cells from live and apoptotic cells).

**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.

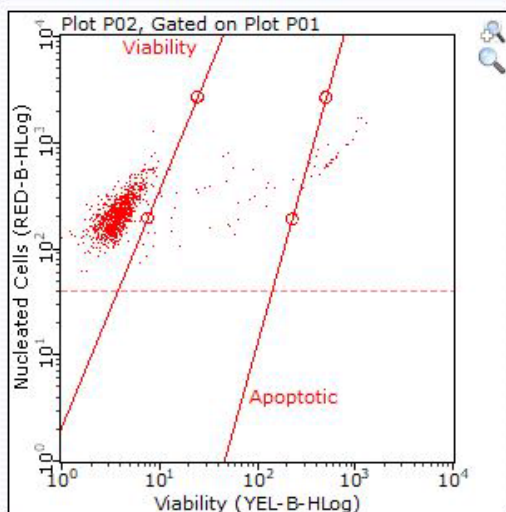


Click and drag bottom of line to move location.

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

- 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.
- 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.

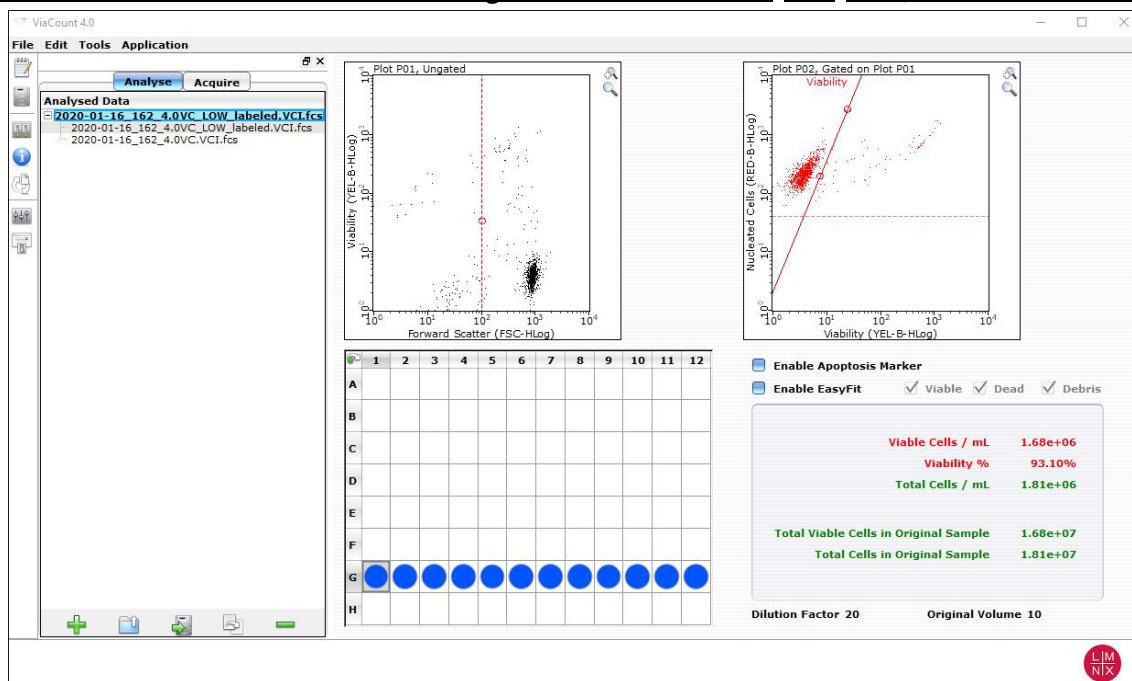
## Open the Utility Program

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

The Guava® ViaCount™ Software Module performs calculations automatically. Results from each run are stored in a data file which can be viewed in the GuavaSoft™ Software and in the corresponding spreadsheet file. Acquired data are displayed in two dot plots: Viability (YEL-B) vs. Nucleated Cells (RED-B) and FSC vs. Viability (YEL-B). Each plot has moveable cursors to allow gating of events. The PO1 dot plot has a marker that allows the users to acquire only events which contain a nucleus or DNA and thus to display live, apoptotic, and dead cells. The PO1 dot plot also has two other markers, one to separate the live from dead cells and a second (optional) one to separate dead from apoptotic cells. The PO2 dot plot has a marker to discriminate cells from debris that might have associated DNA.

The figure below shows typical results for the ViaCount Assay. Jurkat cells were stained with the ViaCount Reagent and acquired on a Guava easyCyte™ System. Live cells appear on the left side of the PO1 plot and at the bottom of the PO2 plot (to the right of the marker). Dead cells are on the right side of the PO1 plot and at the top of the PO2 plot (to the right of the marker). Apoptotic cells are shown in between the live and dead cell populations. This sample of cells also contains some debris (shown as dots to the left of the marker in the PO2 plot).

**Figure 4: Jurkat cells stained with ViaCount Reagent. This culture is at  $1.0^5 \times 10^6$  cells/mL with 93.10% viability.**

For additional information on how to set up the Guava Instrument for acquisition and to adjust the markers in the dot plots, please see the appropriate Guava easyCyte™ System user's guide.

The following results<sup>1</sup> are displayed in the results box after each sample is acquired:

- Number of viable cells per mL
- Percent viability
- Total cells per mL
- Total viable cells in original sample
- Total cells in original sample
- Dilution factor (input value)
- Original volume (input value)

The results<sup>1</sup> of your cell assays are automatically exported<sup>1</sup> to a spreadsheet file. The file contains the following information in a table format for each sample:

- Sample number
- Sample ID
- Cell Concentration Information [Viable cell concentration (cells/mL), Total cell concentration (cells/mL), % Viability]
- Dilution factor
- Original Cell Culture Information [Total viable cells, Total cells, Volume (mL)]

<sup>1</sup> With software containing the optional apoptotic marker, values for % apoptotic and apoptotic cells/mL are also displayed.

The spreadsheet contains additional information from the data acquisition including instrument settings, date stamp, acquisition time, and volume. If the wrong values were input for the Dilution Factor and Original Volume of the cell suspension, this information can be updated during analysis and the data recalculated using the correct values.



The Guava Excel utility program can be used to create that summary spreadsheet in Excel from the csv spreadsheet file. Refer to the appropriate Guava easyCyte System user's guide for how to use this utility.

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## Troubleshooting Tips

1. If using a single-loader (SL) system, mix each cell sample thoroughly on a vortex mixer before acquiring samples for consistent and accurate results. However, avoid vigorous vortexing, which can cause the solution to splash out of the tube or causes cellular damage, resulting in volume loss and erroneous results.  
If using an automated, high-throughput (HT) Guava® System, be sure the mixing option is selected in WorkEdit Software.
2. Multiple acquisitions of a cell sample minimizes sampling error. Statistically, multiple acquisitions yield more accurate cell count and viability results.
3. The default number of events to acquire is 1000. You may input a different number; however, your statistical error will increase as you decrease the number of events for acquisition.
4. If results deviate from expected values, prepare a freshly stained sample and reacquire the data.
5. If the cell count results deviate from expected values, check that the correct values were entered for Dilution Factor and Original Volume of the cell suspension. The ViaCount™ Software Module in GuavaSoft™ Software can be used to recalculate cell counts. Open the data file corresponding to the mistaken entry. Reenter the correct dilution factor or original volume and the cell count values will be recalculated automatically.
6. Ensure that your FSC and Red-Blue threshold settings are set so as not to exclude cellular events.
7. Run Guava easyCheck™ (Cat. No. 4500-0025) to verify proper instrument function and accuracy.
8. Periodically run a Quick Clean cleaning cycle using a water tube (after every 50 sample acquisitions) to prevent a buildup from cell debris in the flow system. If your samples contain significant amounts of cellular debris, run the Quick Clean cycle more often to prevent clogs or blockage.
9. If you are acquiring data from a sample but the Cell Count number is not increasing and the Events to Acquire 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, prepare a new sample 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, salt crystals, or other particulates. Click **Backflush** to flush out the clog into a tube containing 100 µL of bleach. Then run Quick Clean with water to remove bleach residue from the outside of the capillary. If this procedure does not alleviate the problem, consult the appropriate Guava System user's guide or contact Technical Support for additional help.
10. The ViaCount Assay 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 Guava ViaCount Assay with a "clumpy" cell line, such as Chinese Hamster Ovary (CHO) cells, we recommend that you order Guava ViaCount Cell Dispersal Reagent (Cat. No. 4600-0140) to disaggregate the cells. Contact Customer Service or visit our web site at [www.luminexcorp.com](http://www.luminexcorp.com) for detailed information on the Guava ViaCount Cell Dispersal Reagent and assay method.

For more troubleshooting tips, refer to the appropriate Guava easyCyte™ 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="610 722 993 821">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 749">4. Resuspend cells in medium containing 10-20% FBS for 30 min with frequent, gentle agitation. Restain.</li><li data-bbox="1006 749 1377 785">5. Use ViaCount Flex Reagent.</li></ol>

Problem	Possible Cause	Solutions
Cell count is significantly different than expected.	<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>
Guava Excel Utility will not start Microsoft Excel when you try to open a CSV file	<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 Controls

Regardless of the type of cells (adherent or nonadherent) or culture vessel (microplate, tube, or flask) used, each experiment should include the proper negative and positive control samples as indicated below.

- **Negative control sample:** The negative control should be a sample from your cell culture, not treated to induce apoptosis. The stained negative control sample should be run at the beginning of the experiment, and used to adjust the instrument settings for background level staining.
- **Positive control sample:** The positive control should be a sample of apoptotic and dead cells from a culture treated using a known apoptosis induction method for your cell line.

## Prepare Non-Adherent and Adherent Cells

The following protocols describe how to harvest non-adherent or adherent cells cultured in 96-well plates, 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*.

### 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 (Cat 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*.

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## Limitations

1. The results of the assay are dependent upon proper use of reagents and instruments.
2. The Guava® System and ViaCount™ 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 concentrations to within the recommended range. The range for optimal counting results may vary for different cell lines. You may need to adjust the dilution factor accordingly.
3. Cell samples containing cells of a size range between approximately 7 micrometers to 60 micrometers (diameter) will 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.
4. Cell samples containing cell aggregates may not yield accurate cell counts or % viability results. Contact Technical Support or visit one of our web sites for information about Guava ViaCount Cell Dispersal Reagent, a cell disaggregation reagent, optimized for use with the ViaCount Reagent.
5. The ViaCount Assay may not work with all cell lines. Certain cell types may not stain efficiently causing incorrect cell counts and/or viability results. Cell lines expressing fluorescent proteins (for example, transfectants expressing GFP, YFP, etc.) or products (for example, transfectant cell lines expressing non-fluorescent proteins) may yield accurate total cell counts but incorrect viable cell counts. The signal from the expressed transfected fluorescent protein or transfected product may be detected in one of the Guava System fluorescence channels (usually the one detecting stained dead cells). This may cause an inaccurately low viability reading for the culture.

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