

Luminex[®]
complexity simplified.

Guava[®] ViaCount[™] Reagent

For Research Use Only
4600-0010, Rev H
Catalog No. 4000-0040 (40 mL)
Catalog No. 4000-0041 (240 mL)
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Overview

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.

Safety

- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
- 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.
- 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.
- Avoid microbial contamination of the solution, which may cause erroneous results.
- All biological specimens and materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
- Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
- Safety Data Sheets (SDS) for kit reagents are available from our website www.luminexcorp.com

Handling and Storage

- 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.

Materials Provided

- Guava® ViaCount™ Reagent (Cat. No. 4000-0040, 40 mL; Cat. No. 4000-0041, 240 mL)

Materials Required but Not Supplied

- 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
- Bleach
- Deionized water

For High-Throughput (HT) Guava® Systems

- 0.5-mL microcentrifuge tubes (VWR Cat. No. 16466-036 or equivalent) for sample acquisition on the EasyCyte Plus Systems
- 1.5-mL microcentrifuge tubes (VWR Cat. No. 16466-030 or equivalent) for cleaning
- 96-well microplate plates, round bottom (Falcon Cat. Nos. 353910 or 353918) or flat bottom (Falcon Cat. No. 353075 or 353915), or equivalent. Refer to the appropriate Guava® System user's guide for other compatible microplates.
- Reagent reservoirs, 50 mL (VWR Cat. No. 82026-354 or equivalent)

For Single-Loader (SL) Guava® Systems

- 1.5-mL microcentrifuge tubes (VWR Cat. No. 16466-030 or equivalent),
- or 1.2-mL titer tubes (E&K Scientific, Cat. No. 604508-RC or equivalent), for sample acquisition

Additional User Supplied Materials

- Guava® Check Kit (Cat. No. 4500-0020) or 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
- Guava ViaCount™ Flex Reagent (Cat. No. 4700-0060), optional for HT Guava Systems

Protocol

Cell Staining

1. Prepare a uniform cell suspension for counting. See *Cell Sample Preparation* on page 6. 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 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 1×10^4 up to 5×10^5 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
1×10^5 to 1×10^6 cells/mL	10	50 μ L	450 μ L	$<1 \times 10^5$ cells/mL
1×10^6 to 1×10^7 cells/mL	20	20 μ L	380 μ L	$<5 \times 10^5$ cells/mL
$>1 \times 10^7$ cells/mL	40*	20 μ L	780 μ L	$>2.5 \times 10^5$ cells/mL

* 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 μ L of cell suspension into 380 μ L of ViaCount Reagent). If your cell sample shows a Total Cells per mL value of 1×10^7 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.

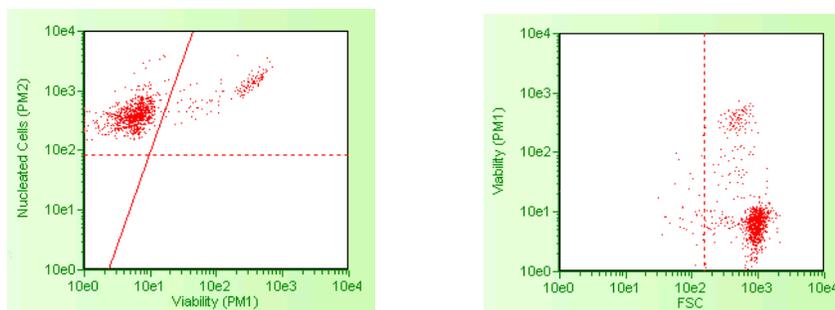
Expected Results

The Guava® ViaCount™ Software Module performs calculations automatically. Results from each run are stored in a data file which can be viewed in the CytoSoft Software and in the corresponding spreadsheet file. Acquired data are displayed in two dot plots: Viability (PM1) vs Nucleated Cells (PM2) and FSC vs Viability (PM1). Each plot has moveable cursors to allow gating of events. The first 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 first 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 second dot plot has a marker to discriminate cells from debris that might have associated DNA.

Figure 1 shows typical results for the ViaCount Assay. Jurkat cells were stained with the ViaCount Reagent and acquired on a Guava PCA System. Live cells appear on the left side of the first plot and at the bottom of the second plot (to the right of the marker). Dead cells are on the right side of the first plot and at the top of the second 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 second plot).

Figure 1: Jurkat cells stained with ViaCount Reagent. This culture is at $1.0^5 \times 10^6$ cells/mL with 85.5% 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 ViaCount Assay chapter and, where applicable, Appendix A in the appropriate Guava System user's guide.

The following results* 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* of your cell assays are automatically exported 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)]

* 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 System user's guide for how to use this utility.

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, 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 CytoSoft 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 PM2 threshold settings are set so as not to exclude cellular events. Refer to the ViaCount chapter of the GuavaSoft™ Modules User's Guide for more information.
7. Run Guava Check (Cat. No. 4500-0020) or 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 for detailed information on the Guava ViaCount Cell Dispersal Reagent and assay method.

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

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 1×10^4 to 5×10^5 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 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.

Cell Sample Preparation

Preparing 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.

Preparing 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.

Preparing non-adherent cells

1. Set up initial culture conditions, such that after culture and treatment, cells are at a concentration of 1×10^5 to 1×10^7 cells/mL in serum- or albumin containing medium.
2. Proceed to *Cell Staining*.

Preparing adherent cells

For harvesting adherent cells, use your method of removal. Reagents such as EDTA or trypsin can be used to dissociate the cells from the flask and should create single-cell suspensions. If using mechanical means to dislodge the cells, 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 1×10^5 to 1×10^7 cells/mL.
3. Proceed to *Cell Staining*.

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