

Guava® ViaCount® Reagent

Catalog No. 4000-0040 (100 Tests)

Catalog No. 4000-0041 (600 Tests)

For Rapid Cell Count and Viability Determinations

Research Use Only
Not for Use in Diagnostic Procedures

1. PRODUCT DESCRIPTION AND INTENDED USE _____

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 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.

2. MATERIALS PROVIDED _____

Guava ViaCount Reagent (Cat. No. 4000-0040, 100 tests/bottle; Cat. No. 4000-0041, 600 tests/bottle)

3. HANDLING AND STORAGE _____

1. 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.
2. The Guava ViaCount Reagent contains light-sensitive dyes. Shield from excessive exposure to light.

4. WARNINGS AND PRECAUTIONS _____

1. The Guava ViaCount Reagent is intended for research use only.
2. Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling this reagent.
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. A Material Safety Data Sheet (MSDS) for the reagent is available from our web site (www.guavatechnologies.com) or by contacting Guava Customer Support (support@guavatechnologies.com).
4. The Guava ViaCount Reagent contains sodium azide, which is toxic. Contact with acids liberates toxic gas. Flush plumbing with copious amounts of water when disposing of azide compounds to avoid potentially explosive conditions arising from azide deposits in pipes.
5. Avoid microbial contamination of the solution, which may cause erroneous results.
6. All biological specimens and materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.

7. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.

5. EQUIPMENT AND MATERIALS REQUIRED _____

- Guava system with CytoSoft software containing the ViaCount module
- Guava ViaCount Reagent
- 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
- For the Guava PCA™ and Guava EasyCyte™ Mini systems:
 - Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Cat. No. 16466-030, or equivalent) or
 - Sample acquisition tubes, 1.2 mL titer tubes (E&K Scientific, Cat. No. 604508-RC, or equivalent)
- For the Guava PCA-96, Guava PCA-96 AFP, and Guava EasyCyte systems:
 - Microcentrifuge tubes with screw caps, 0.5 mL (VWR, Cat. No. 16466-036) for sample acquisition
 - Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Cat. No. 16466-030, or equivalent) for washing
- Guava ViaCount CDR™ (Cat. No. 4700-0050), optional
- Vortex mixer
- Disposable gloves
- 20% bleach solution
- Guava ICF instrument cleaning fluid (Cat. No. 4200-0140), optional
- Deionized water

6. REAGENT AND SAMPLE PREPARATION _____

1. Prepare a uniform cell suspension for counting. 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 onto 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.

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 up 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 of concentrations $> 2 \times 10^7$, 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.

NOTE: If using CytoSoft 1.2 or lower, prepare an unstained cell sample for setting instrument thresholds by diluting the cells in dilution buffer, such as PBS, in a sample tube. Use the same dilution factor you used for the stained sample.

7. EXPECTED RESULTS _____

The Guava ViaCount application 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 2 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. 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).

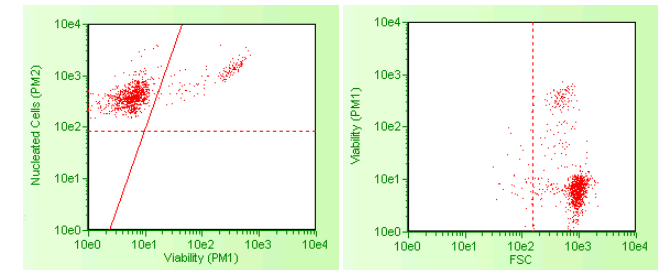


Figure 1 Jurkat cells stained with ViaCount. This culture is at 1.05×10^6 cells/mL with 85.5% viability.

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 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.

If using CytoSoft 1.2 or lower, a summary spreadsheet in Excel is automatically created for every ViaCount data file. If using CytoSoft 2.0 or higher, 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.

8. TROUBLESHOOTING TIPS

1. If using the PCA or EasyCyte Mini, 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 the Guava PCA-96, PCA-96 AFP, or EasyCyte, be sure the mixing option is selected in WorkEdit software.
2. If using CytoSoft 1.2 or lower, and the original cell suspension is highly concentrated (much greater than 1×10^7 cells/mL), dilute the unstained cell sample 1:100 with dilution buffer to set the instrument threshold.
3. Multiple acquisitions of a cell sample minimizes sampling error. Statistically, multiple acquisitions yield more accurate cell count and viability results.
4. 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.
5. If results deviate from expected values, prepare a freshly stained sample and reacquire the data.
6. 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 application in CytoSoft (CytoAnalysis, if using CytoSoft 1.2 or lower) 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.

7. Run Guava Check using the Guava Check kit (Cat. No. 4500-0020) to verify proper instrument function and accuracy.
8. Periodically run Quick Clean 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 μ L of sample in the tube. If not, add additional buffer to bring the volume up to 100 μ L or proceed to the next sample. If the sample volume is greater than 100 μ L, then the lack of events is probably due to a clog. A clog or blockage can be caused by cell aggregates, cell debris, bleach crystals, or other particulates. Click **Backflush (Purge)**, if using CytoSoft 1.2 or lower) to flush out the clog into a tube containing 20% bleach. Then run Quick Clean to remove bleach residue. If this procedure does not alleviate the problem, consult the appropriate Guava system user's guide or contact Technical Support for additional help.
10. ViaCount 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 CDR (Cat. No. 4600-0140) to disaggregate the cells. Contact Customer Service or visit our web site at www.guavatechnologies.com for detailed information on the Guava ViaCount CDR reagent and assay method.

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

9. LIMITATIONS

1. The results of the assay are dependent upon proper use of reagents and instruments.
2. If using CytoSoft 1.2 or lower, the Guava PCA may not measure the % Viability of low viability cell suspensions as accurately as for high viability samples. In general, the actual viability of cell suspensions with <30% live cells may be lower than reported by ViaCount.
3. 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.
4. Cell samples containing cells of a size range between approximately 7 microns to 60 microns (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 Guava Technical Support for more information.
5. Cell samples containing cell aggregates may not yield accurate cell counts or % viability results. Contact Guava Technical Support or visit our web site for information about Guava ViaCount CDR, a cell disaggregation reagent, optimized for use with the ViaCount Reagent.
6. 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.

10. DISCLAIMER OF WARRANTY

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

11. RETURN GOODS POLICY

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

12. TRADEMARKS AND PATENTS

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