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Guava® ViaCount™ CDR Cell Dispersal Reagent Package Insert

Catalog No. 4700-0050 (100 tests)

For Cell Count and Viability Determination of Cell Cultures with Aggregates

Research Use Only
Not for Use in Diagnostic Procedures

DESCRIPTION

The Guava® ViaCount™ assay provides absolute cell count and viability data on cell suspensions from a variety of cultured mammalian cell lines. However, cell samples containing cell aggregates may not yield accurate and consistent results with Guava ViaCount reagent alone. The differential staining pattern of viable and non-viable cells in clumps are difficult to resolve and assess individually. Cell aggregates may clog or be excluded from the flow cell, hampering the assay.

The ViaCount Cell Dispersal Reagent is an enzymatic reagent formulated to gently disaggregate the cells in clumps, yielding a more uniform cell suspension for assay. This assay has been optimized for Chinese Hamster Ovary (CHO-K1) cell lines that have been adapted to suspension culture and tend to form cell aggregates. However, Guava ViaCount CDR may be useful for disaggregating other types of clumpy suspension cultures, yielding improved consistency of counts in the ViaCount application.

After treatment with Guava ViaCount CDR, the homogeneous cell suspension is stained with Guava ViaCount reagent to distinguish between viable and non-viable cells by the differential permeability of DNA-binding dyes in the Guava ViaCount reagent. The fluorescence of each dye is resolved operationally on the Guava System to allow the quantitative assessment of both viable and non-viable cells in the suspension. The results are displayed in a dot plot, showing Nucleated Cells vs Viability, and in summary data boxes. Results are automatically exported to a spreadsheet file. You can review and reanalyze data files using GuavaSoft™ software or an FCS-compatible program if you choose the option to save FCS 3.0 files.

Guava ViaCount CDR can also be used to remove adherent cells from culture vessels as part of the sample preparation protocol for other Guava assays. Refer to the appropriate Guava assay package insert for instructions.

MATERIALS PROVIDED

- Guava® ViaCount™ CDR (Catalog. No. 4700-0050, five 1.0-mL vials)

REAGENT STORAGE, HANDLING, AND STABILITY

1. Store Guava® ViaCount™ CDR frozen at –20°C upon receipt. The expiration date on the package label refers to the frozen reagent. Do not use the reagent after the expiration date.

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. Guava ViaCount CDR is a sterile solution. Avoid microbial contamination, which may cause erroneous results.
4. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
5. 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.*
6. Do not pipette by mouth.
7. 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.
8. 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.
9. Follow your institution's safety procedures for working with chemicals and handling biological samples.
10. In the event of damage to the protective packaging, consult the Safety Data Sheet (SDS) for instructions.
11. Safety Data Sheets (SDS) for kit reagents are available by contacting Luminex Corporation or visiting our website at www.luminexcorp.com

MATERIALS REQUIRED BUT NOT PROVIDED

- Guava® Instrument with GuavaSoft™ software
- Guava® ViaCount™ Reagent (Catalog. No. 4000-0040 or equivalent)
- 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.
- Fetal bovine serum, optional
- Micropipettors
- Disposable micropipettor tips
- Microcentrifuge tubes with screw caps, 1.5 mL (VWR, Catalog. No. 16466-030, or equivalent) or
- Vortex mixer
- 37°C incubator
- Tube rotator
- Disposable gloves
- 100% Bleach solution containing between 5-6% hypochlorite
- Deionized water
- Guava® ICF Instrument Cleaning Fluid (Catalog. No. 4200-0140)

REAGENT AND SAMPLE PREPARATION

In this protocol, the cell culture sample is mixed with an aliquot of the Cell Dispersal Reagent and ViaCount reagent, then incubated at 37°C on a rotator for up to 30 minutes. During this incubation period, the cell clumps disaggregate by enzymatic action. After the incubation, you add ViaCount reagent again to stain the cells more completely for cell counting and viability assessment.

We have found that incubating 2 to 3 x 10^6 cells/mL suspension-cultured CHO-K1 cells using a 0.8x concentration of Guava® ViaCount CDR for 20 or 30 minutes provides fairly consistent results for CHO-K1 cells from cultures of 50-100% viability and between 5x 10^5 to 1 x 10^7cells/mL density. However, you may need to adjust the concentration of ViaCount CDR or adjust the incubation time for disaggregation to suit your specific CHO-K1 clone. The enzymatic treatment preferentially digests dead cells and dying cells. Optimization of this assay involves balancing the efficiency of enzymatic clump disaggregation against the digestive loss of the dead cells. Cell samples containing many large cell aggregates may require a higher concentration (0.8x to 1x) of Guava ViaCount CDR or longer incubation times to effectively break up the aggregates. Lower concentrations of ViaCount CDR (0.2x to 0.8x) or shorter incubation times may yield more accurate results for cell samples with less aggregation or of low viability (<70%). Adding up to 5% fetal bovine serum in the cell sample medium helps protect dead cells from digestion.

Assay Procedure

1. Thaw the Cell Dispersal Reagent. Bring the Guava® ViaCount™ reagent to room temperature.
2. Prepare a dilution of Guava ViaCount CDR, as desired. (For example, mix 1 mL of ViaCount CDR with 0.25 mL of PBS buffer to yield 1.25 mL of a 0.8x working solution.)
3. Obtain well-mixed CHO-K1 cell samples for assay. Cell samples should be at a concentration of 5 x 10^5 to 5 x 10^6 cells/mL for the assay. If your cell samples are more concentrated, dilute them using PBS dilution buffer to bring the cell concentration to this range.
4. Disaggregate and stain the cell samples by mixing 50 µL of cells, 50 µL of 0.8x Cell Dispersal Reagent, and 150 µL of Guava ViaCount reagent in a 1.5-mL microcentrifuge tube. Cap the tube and vortex gently to mix.
5. Place sample tubes on a rotator in an incubator at 37°C. Incubate tubes for 20 to 30 minutes.
6. Add 250 µL of Guava ViaCount reagent to each tube and incubate for an additional 5 minutes at room temperature.
7. Disaggregated cell samples are ready for acquisition. For best results, acquire each sample within 10 minutes after the final incubation in Guava ViaCount reagent (step 6).

EXPECTED RESULTS

The Guava® ViaCount™ CDR assay yields consistent cell count and viability results for CHO-K1 cell samples, which tend to aggregate in suspension culture. When comparing Guava ViaCount CDR assay results to other manual staining and counting methods (for example, hemacytometer counts on cells stained with ethidium bromide and acridine orange), you may find an average of 20% difference between the Guava ViaCount CDR assay and the manual method for Total Cells per mL and Live Cells per mL results. The average difference in %Viability results between the Guava ViaCount CDR assay and the manual method is 5%. Often the Guava ViaCount cell count results are lower than the manual count results. Trypan blue and ethidium bromide/ acridine orange viability stains do not discriminate between healthy, live cells

and early apoptotic cells. However, the ViaCount reagent can resolve these populations. For healthy cultures (>90% viable), the difference between Guava ViaCount results and manual counts is small (<10% difference); however, for less robust cultures, the difference can be much greater due to the increased number of apoptotic cells. In addition, the Cell Dispersal Reagent digests dead cells preferentially, which can affect the Total Cell count and %Viability results. The potential for decreasing the Total Cell count and increasing the %Viability result is more probable for cultures of low viability (<80%).

Acquired data are displayed in a dot plot, which shows fluorescence of all Nucleated Cells vs Viability. Figure 1 shows an example of staining results obtained using the Guava ViaCount CDR assay. CHO-K1 cells grown in suspension culture were incubated with 0.8x Cell Dispersal Reagent and ViaCount reagent for 20 minutes at 37°C on a rotator, then stained with additional ViaCount reagent as described in Section .

- Viable, healthy cells appear to the left of the marker (positive for nuclear stain and negative for dead cell stain).
- Dying and apoptotic cells often appear as an adjacent population to the right of the live cells (positive for nuclear stain and dim for dead cell stain).
- Dead cells appear to the right of the dying and apoptotic cells and should be well resolved from the live cell population (positive for nuclear stain and positive for dead cell stain).

You can move and tilt the gating marker to discriminate live cells from dying and dead cells for more accurate gating of events.

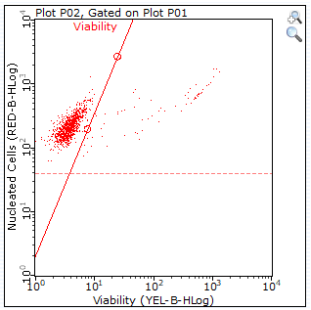

Figure 1. Data from CHO-K1 cells treated with the Cell Dispersal Reagent and ViaCount reagent, acquired on a Guava System.

The Guava ViaCount application performs calculations automatically. The results are displayed as follows in the results box after each sample is acquired:

- Number of Viable Cells per mL
- %Viability, Total Cells per mL
- Total Viable Cells in Original Sample
- Total Cells in Original Sample
- Dilution Factor (input value)
- Original Volume (input value)

For additional information regarding the Guava ViaCount application see the appropriate Guava system user's guide.

TROUBLESHOOTING TIPS

1. Mix each cell sample thoroughly on a vortex mixer before acquiring samples for consistent and accurate results.
2. If the concentration of the stained cell sample for data acquisition is high ($\geq 5 \times 10^5$ cells/mL), the Guava® System may not yield accurate results. Pre-dilute the sample with PBS buffer to bring the cell concentration into an acceptable range. If you have already performed the Guava® ViaCount™ CDR assay on this sample, add additional ViaCount reagent to dilute the cell sample further and reacquire data.
3. Acquire data on disaggregated, stained cells soon after staining. Dilution of the Cell Dispersal Reagent with ViaCount reagent slows, but does not stop, enzymatic treatment of the cells. Prolonged exposure of the cells to Guava ViaCount CDR may result in low Total Cell counts and high % Viability values, due to preferential digestion of dead and dying cells. For best results, acquire the samples within 10 minutes after the final ViaCount staining step. Keep samples protected from light until data acquisition begins.
4. For cell samples of lower viability (<80%), adding up to 5% fetal bovine serum in the cell culture medium can help preserve dead cells from enzymatic damage and loss, improving Total Cell count and %Viability accuracy.
5. Periodically run Quick Clean using deionized water (after every 20 to 25 sample acquisitions) to prevent a buildup from cell debris in the flow system. If your samples contain significant amounts of cellular debris, run Quick Clean more often using Guava ICF followed by water, to prevent clogs or blockage.
6. A clog or blockage of the flow system can be caused by cell aggregates, cell debris, bleach crystals, or other particulates. If you are acquiring data from a sample but the Cell Count number is not increasing and the Events to Acquire progress bar is not moving, there is probably a blockage of the flow system. Load a 20% bleach tube, then click Backflush to flush out the clog. Load a tube of deionized water, 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 the package insert for Guava ICF, or contact Luminex Technical Support for additional help.

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® ViaCount™ CDR Assay has been tested for use on CHO-K1 suspension cultures at 50 to 100% viability. The assay has not been validated for use on cultures that are less than 50% viable.
3. The Guava System yields optimal results when the stained cell sample for acquisition is between 1×10^4 and 5×10^5 cells/mL. To obtain the most accurate assay results, adjust the concentration of the cell samples to within the recommended range.
4. Cell samples containing cells of a size range between approximately 2 to 60 microns (diameter) yields the most accurate results on the Guava system. If your cell samples contain significant amounts of cells outside this size range, call Luminex Technical Support for more information.
5. Fetal bovine serum inhibits Guava ViaCount CDR at concentrations greater than 5% in the culture medium. If your culture medium contains serum, you must dilute the cell samples with PBS to bring the serum concentration to 5% or lower before using them in the assay.
6. The Guava ViaCount CDR assay may not work with all cell lines. Certain cell types may not disaggregate efficiently with the Cell Dispersal Reagent, or stain effectively with the ViaCount reagent, causing incorrect cell counts and/or viability results. Cell lines expressing fluorescent proteins (for example, transfectants expressing GFP, YFP, etc) or other products (for example, transfectant cells lines expressing non-fluorescent proteins) may yield accurate total cell counts but incorrect viable cell counts. Background 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 can cause an inaccurately low viability reading for the culture.
7. Guava ViaCount CDR is formulated to meet most CHO-K1 assay requirements. Modification of the assay protocol and reagent concentration(s) may be necessary to ensure optimal performance for individual cell assays. Contact Luminex Technical Support for suggestions and additional information.

TECHNICAL SUPPORT

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TRADEMARKS