

Guava® EasyCyte™ CellToxicity™ KitCat. No. 4500-0230
100 Tests**For cell-mediated cytotoxicity measurements****Research Use Only
Not for Use in Diagnostic Procedures****1. PRODUCT DESCRIPTION AND INTENDED USE _____**

One of the functions of the immune system is to recognize and destroy targets, such as tumor cells or cells infected with microbes (bacteria or viruses). This is accomplished by effector cells, such as T cells, NK cells, macrophages, or granulocytes, which employ cell-mediated cytotoxicity (CMC), natural killer activity (NK), or antibody-dependent cellular cytotoxicity (ADCC), respectively. CMC and NK activities are commonly monitored to assess the potency of immune cell stimulation by small molecules, antibodies, or other regulatory molecules, whereas ADCC activity is used to assess the potency of antibodies for therapy.

Cellular cytotoxicity has traditionally been measured using a ⁵¹Chromium release assay,¹ although alternative assays (including LDH,² MTT,³ and alamarBlue™,⁴ among others) have been used recently due to concerns about the handling and disposal of radioactive materials. Another approach to measure cytotoxicity involves the detection and quantification of dead target cells by flow cytometry. Live and dead target and effector cells can be distinguished through the use of differential staining by fluorescent dyes.⁵⁻⁸

The Guava CellToxicity assay uses two dyes: a cell permeant painting dye and a cell impermeant DNA binding dye, to distinguish effector or cytotoxic cells from target cells, and to distinguish dead target cells from live target cells. The cell painting dye, carboxyfluorescein diacetate succinimidyl ester or CFSE (also known as CFDA SE), diffuses freely into cells where intracellular esterases cleave off the acetate groups, converting it to a fluorescent, membrane-impermeant dye. The dye is retained in the target cell's cytoplasm even after the cell is killed due to covalent crosslinking to proteins through its succinimidyl groups. Painted target cells are subsequently added to unpainted effector cells at various ratios and incubated to allow effector cells to function. A dead cell dye, 7-AAD⁹, is then added to determine which of the painted target cells have been killed.

2. MATERIALS PROVIDED _____

Guava EasyCyte CellToxicity Kit (Cat. No 4500-0230) includes the following reagents:

- Guava CFSE (Part No. 4700-0130, 100 µg)
- CellToxicity 7-AAD (Part No. 4700-0150, 4 x 1 mL vial)
- Anhydrous DMSO (Part No. 4300-0260, 2.0 mL)

3. HANDLING AND STORAGE _____

1. Always store Guava CFSE reagent (either powder or in solution) at (minus) –10° to –30°C in a desiccator.
2. Avoid repeated freezing and thawing of the reconstituted Guava CFSE reagent. Guava recommends that the reagent be aliquotted into smaller volumes to eliminate multiple freeze/thaw cycles.

3. The Guava CFSE reagent is light sensitive. Shield from excessive exposure to light.
4. Store Guava CFSE reconstituted in DMSO at (minus) –10° to –30°C up to 3 months. After 3 months, Guava CFSE reconstituted in DMSO is no longer stable.
5. Refer to expiration dates on component labels. Guava recommends using all kit components before the expiration date.

4. WARNINGS AND PRECAUTIONS _____

1. The Guava EasyCyte CellToxicity Kit is intended for research use only.
2. 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. Avoid specimen contact with skin and mucous membranes.
3. If using a frozen aliquot of stock Guava CFSE Painting Solution, make sure it is completely thawed before use by repeat pipetting.
4. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
5. Avoid microbial contamination of the solutions, which may cause erroneous results.
6. Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this product.
7. Dispose of all materials using proper precautions in accordance with federal, state, and local regulations.
8. Material Safety Data Sheets (MSDS) for kit reagents are available from our web site (www.guavatechnologies.com) or by contacting Guava Technologies Technical Support (support@guavatechnologies.com telephone 866-448-2827 or 510-576-1400).
9. The Guava EasyCyte CellToxicity Kit contains DMSO, which may transport toxic compounds directly through the skin. Avoid prolonged or repeated exposure.
10. The Guava EasyCyte CellToxicity Kit contains 7-AAD, which is a potential carcinogen. Avoid contact with skin and eyes. Wear suitable protective clothing, gloves, and eye/face protection.

5. EQUIPMENT AND MATERIALS REQUIRED _____

- Guava EasyCyte system with CytoSoft™ software that includes the CellToxicity assay module
- Guava EasyCyte CellToxicity Kit (Cat. No. 4500-0230)
- Effector cell suspensions [such as NK cells, cloned T cells, peripheral blood mononuclear cells (PBMC)]
- Target cell suspensions (such as K562 or YAK cells, or allogeneic PBMC)
- 1X phosphate buffered saline (PBS), pH 7.2 to 7.4, or equivalent
- Triton X-100 (Sigma Cat. No. X100) or NP40 (Calbiochem, Cat. No. 492015), or equivalent
- Micropipettors, single- or multi-channel, as needed
- Disposable micropipettor tips
- 96-well microplates, round bottom (Falcon Cat. No. 353910 or 353918, or equivalent). Refer to the *Guava EasyCyte System User's Guide* for other compatible round bottom microplates.
- Centrifuge with 96-well microtiter plate holders
- For adherent cells only: hydrogel-coated flat bottom microplates (Costar Cat. No. 3474, or equivalent.)
- Microcentrifuge tubes, 1.5 mL (VWR, Cat. No. 20170-201, or equivalent)

- 0.5-mL microcentrifuge tubes (VWR Cat. No. 20170-210, or equivalent) for sample acquisition
- Brown vials, 0.5 mL (VWR Cat. No. 20170-724, or equivalent)
- Reagent reservoirs, nonsterile, 55 mL (VWR Cat. No. 21007-970, or equivalent) or sterile, 100 mL (VWR Cat. No. 20901-921, or equivalent)
- Vortex mixer
- Disposable gloves
- 37°C CO₂ incubator
- Guava ViaCount® reagent (Cat. No. 4000-0040), or Guava PCA-96 ViaCount® Flex reagent (Cat. No. 4700-0060), optional
- Deionized water
- Guava ICFT™ Instrument Cleaning Fluid (Cat. No. 4200-0140), optional
- 20% bleach solution
- Guava ViaCount CDR™ (Cell Dispersal Reagent) for adherent cells (Cat. No. 4700-0050), optional

6. REAGENT AND SAMPLE PREPARATION _____

Recommended Assay Conditions: For non-adherent cells, Guava recommends using 2.5 x 10⁶ cells/mL when painting target cells. This corresponds to approximately 25 tests at 500 cells/µL in 200 µL for each milliliter of cells painted. If a lower concentration of cells is used per test, more tests can be performed and if a higher volume is used per test, fewer tests can be performed. For adherent cells, Guava recommends painting cells when they are approximately 80% confluent. In a T75 cm² flask of CHO cells, this corresponds to approximately 1.0 x 10⁷ cells, which is sufficient for 100 tests at 500 cells/µL in a 200-µL volume.

The Guava CellToxicity assay should be set up in round bottom, 96-well microplates to allow sufficient contact between effector and target cells. However, if target cells are adherent, use hydrogel-coated, flat bottom, 96-well plates to prevent reattachment of the target cells. In that case, in order to see sufficient killing of targets, the effector to target cell ratios used and/or the total number of cells per well added might have to be increased or the samples incubated for longer periods.

Time Considerations: The process of painting the target cells with the Guava EasyCyte CellToxicity Kit and running on the Guava EasyCyte usually takes 6 hours if doing a standard 4-hour incubation of effector cells with target cells. Preparation of effector cells could take from 15 minutes (if just harvesting cloned effector cells) to 1 hour (if isolating PBMC from whole blood) to 1 week (if priming allogeneic effector cells). Acquiring data on the Guava EasyCyte system usually takes approximately 30 seconds per sample, but it depends on the final cell concentration.

Reagent Preparation**0.1 mM Stock Guava CFSE Painting Solution**

1. Add 1794 µL anhydrous DMSO to the vial containing 100 µg of Guava CFSE.
2. Mix thoroughly by repeatedly inverting the vial to ensure all solid is dissolved.
NOTE: The amount of solid CFSE per vial is small and may be hard to see.
3. Aliquot 100 to 250 µL of the resuspended Guava CFSE into brown vials and store at (minus) –10° to –30°C, desiccated until needed.

NOTES: Guava CFSE is stable for 3 months at (minus) –10° to –30°C when reconstituted in DMSO. Avoid more than two freeze/thaw cycles of the reconstituted Guava CFSE since the solvent takes up moisture and causes the dye to break down.

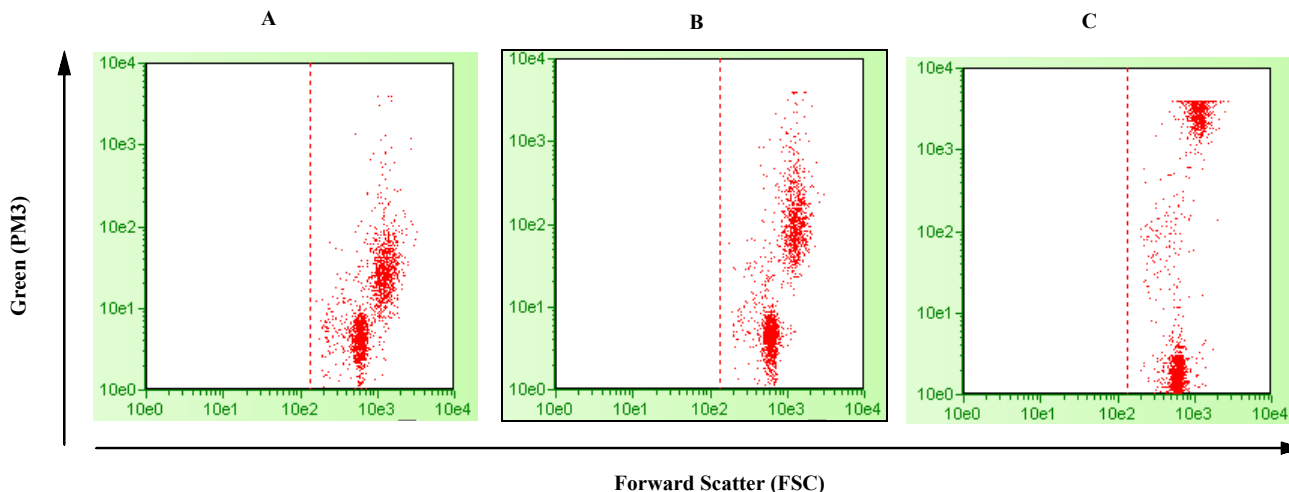


Figure 1 Guava CFSE painted target cells mixed with an equal number of unpainted effector cells.

A) Results obtained when the Guava CFSE concentration was too low. While there is a separation between the two populations, it is not optimal (MFI ratio is less than 20).

B) Results obtained when the Guava CFSE concentration was correct. Notice there is a very distinct separation between the painted and unpainted populations and that the MFI ratio is 50.

C) Results obtained when the Guava CFSE concentration was too high and the stained population is saturated (MFI ratio greater than 250).

To determine the optimal Guava CFSE concentration, the instrument was adjusted so that the unpainted effector cells were evenly distributed between 10e0 and 10e1 on the Green (PM3) scale.

Cell Preparation

For optimal painting, cells should be in log-phase growth.

Determining Optimal Cell Painting Concentration

Each cell line might require slightly different optimal painting concentrations. We have found that 0.25 μM Guava CFSE works best for K562 and Daudi cell lines, as well as for PBMCs. However, you may need to determine the optimal concentration for painting your cell lines. We recommend that you start with a concentration of between 0.0625 and 0.5 μM Guava CFSE, and then titer the reagent either up or down as appropriate by factors of two. Optimal results are obtained when you ensure that there is a 40 to 120-fold difference in the mean fluorescence intensity (MFI) between the painted target cells and the unpainted effector populations, and that the painted population is not saturating. See Figure 1 for examples of correct and incorrect target cell painting patterns.

Cell Painting Protocol

For Non-Adherent Cells

1. Remove cells from culture and place in a conical tube. For cells from a T75 cm^2 flask, use a 50-mL conical tube.
2. Count cells using Guava ViaCount reagent (for Guava PCA system) or Guava PCA-96 ViaCount Flex (for Guava EasyCyte system). Refer to Guava ViaCount or Guava PCA-96 ViaCount Flex package inserts for suggested protocols.
3. Centrifuge the cells at 300 \times g for 5 to 7 minutes.
4. Aspirate the culture media being careful not to disturb the cell pellet.
5. Add 1 mL PBS per 2.5×10^6 cells.

IMPORTANT NOTE: Resuspend cells in PBS only. Do not add any protein to the PBS. Protein in the PBS will cause incomplete painting of the cells.

6. Resuspend cells gently by repeat pipetting.
7. Centrifuge cells at 300 \times g for 5 to 7 minutes.
8. Aspirate the PBS from the tube.
9. Add 1 mL PBS per 2.5×10^6 cells.

IMPORTANT NOTE: Paint the cells at 2.5×10^6 cells/mL to determine the appropriate concentration of Guava CFSE. Changes in the cell concentration can change the amount of dye needed for optimal painting.

10. Gently resuspend cells by repeat pipetting. Avoid generating bubbles.

11. Add stock Guava CFSE Painting Solution as determined from your staining optimization study. See Table 1 for suggested volumes.

IMPORTANT NOTE: Ensure aliquotted stock Guava CFSE Painting Solution is completely thawed before use by repeat pipetting.

Table 1. Volume (in μL) of Stock Guava CFSE Painting Solution per Volume of Non-Adherent Cells (in mL)

Final Guava CFSE Concentration (μM)	For 1 mL cell suspension (25 tests)	For 2 mL cell suspension (50 tests)	For 4 mL cell suspension (100 tests)	For 5 mL cell suspension (125 tests)	For 10 mL cell suspension (250 tests)
0.125	1.25	2.50	5.0	6.25	12.5
0.25	2.50	5.00	10.0	12.50	25.0
0.5	5.00	10.00	20.0	25.00	50.0

12. Incubate cells in a loosely capped tube at 37°C in CO_2 for 15 minutes.
13. After incubation, centrifuge the cells at 300 \times g for 5 to 7 minutes.
14. Aspirate the Guava CFSE in PBS from the tube.
15. Add 1 mL culture media with at least 10% serum per 2.5×10^6 cells and resuspend by repeat pipetting.

IMPORTANT NOTE: Use culture media with at least 10% serum to quench any remaining unbound dye.

16. Incubate cells in a loosely capped tube at 37°C in CO_2 for 30 minutes.
17. After incubation, centrifuge the cells at 300 \times g for 5 to 7 minutes.
18. Aspirate the media from the tube.
19. Add 2 mL PBS per 2.5×10^6 cells, and then resuspend by gently vortexing.
20. Centrifuge the cells at 300 \times g for 5 to 7 minutes.
21. Aspirate the PBS from the tube.
22. Repeat steps 19 through 21 two more times for a total of three washes with PBS.
23. After the last wash, resuspend the cells in medium with serum used for the cytotoxicity assay.

For Adherent Cells

1. Grow adherent cells until they are approximately 80% confluent.

IMPORTANT NOTE: Paint the cells at approximately 80% confluency to determine the appropriate concentration of Guava CFSE. Changes in the cell confluency can change the amount of dye needed for optimal painting.

2. Aspirate the culture media from the flask.
3. To a T75 cm^2 flask, add 25 mL PBS, and then gently tilt the flask to rinse the cells. If using another flask size, scale all volumes based on the ratio of the flask surface area to that of a T75 cm^2 flask. For example, if growing cells in a T25 cm^2 flask, use 8.3 mL of PBS, and if in a T150 cm^2 flask, use 50 mL of PBS.
4. Aspirate the PBS from the flask.
5. Add 25 mL PBS.

IMPORTANT NOTE: Add only PBS to the flask. Do not add any protein to the PBS. Protein in the PBS will cause incomplete painting of the cells.

6. Add stock Guava CFSE Painting Solution as determined from your staining optimization study. See Table 2 for suggested volumes.

IMPORTANT NOTE: Ensure aliquotted stock Guava CFSE Painting Solution is completely thawed before use by repeat pipetting.

Table 2. Volume (in μL) of Stock Guava CFSE Painting Solution per Type of Tissue Culture Flask Containing Adherent Cells

Final Guava CFSE Concentration (μM)	For T25 cm^2 Flask (approx. 33 tests)	For T75 cm^2 Flask (approx. 100 tests)	For 150 cm^2 Flask (approx. 200 tests)
0.125	10.4	31.25	62.5
0.25	20.8	62.50	125.0
0.5	41.7	125.0	250.0

7. Incubate cells in a vented, capped flask at 37°C in CO_2 for 15 minutes.
8. Aspirate the Guava CFSE in PBS from the flask.
9. Add 25 mL culture media with at least 10% serum to the flask.

IMPORTANT NOTE: Use culture media with at least 10% serum to quench any remaining unbound dye.

10. Incubate cells in a vented capped flask at 37°C in CO₂ for 30 minutes.
11. After incubation, aspirate the media from the flask.
12. Add 10 mL PBS, and then gently tilt the flask to rinse the cells.
13. Aspirate the PBS from the flask.
14. Detach the cells from the plastic substrate with Guava ViaCount CDR. Alternatively, you may use trypsin, EDTA, or mechanical means to detach the cells, whichever is appropriate.
 - a. Dilute the Guava ViaCount CDR 1:3 with PBS.
 - b. Add 5 mL of diluted Guava ViaCount CDR and incubate in a 37°C incubator for 3 to 5 minutes or until cells start to detach.
 - c. Add 5 to 10 mL of media with at least 5% serum, and then pipet repeatedly to release cells from the flask.
15. Transfer cells to a 50 mL conical tube.
16. Centrifuge cells at 300 x g for 5 to 7 minutes.
17. Aspirate the culture media from the tube being careful not to disturb the cell pellet.
18. Add 2 mL PBS per 2.5 x 10⁶ cells to the tube, and then resuspend by gently vortexing.
19. Centrifuge the cells at 300 x g for 5 to 7 minutes.
20. Aspirate the PBS from the tube.
21. Repeat steps 18 through 20 two more times for a total of three washes with PBS.
22. After the last wash, resuspend the cells in medium with serum used for the cytotoxicity assay.

Preparing Effector Cells

Effector cell preparation depends on the particular type of assay (NK, CMC, ADCC) and on each investigator's choice of cells. Guava recommends preparing effector cells as you normally would for other cytotoxicity assays, and then following the instructions in this insert for adjusting the effector cell concentration and mixing the effector cells with target cells.

Mixing Painted Target and Unpainted Effector Cells

1. Determine the total cell concentration of painted target cells and unpainted effector cell preparations using Guava ViaCount reagent (for Guava PCA system) and Guava PCA-96 ViaCount Flex reagent (for Guava EasyCyte system). Refer to the Guava ViaCount and Guava PCA-96 ViaCount Flex package inserts for protocols.

IMPORTANT NOTE: Determine the cell concentration after painting, because there can be significant cell loss during the wash steps. The painted cells will all appear as dead cells because the Guava CFSE dye is detected in the PM1 (Viability) parameter.

2. Adjust the cell concentrations of both populations to the desired concentration in medium with serum. The Guava EasyCyte system is designed to run samples at concentrations from 1.0 x 10⁴ to 5.0 x 10⁵ cells/mL. However, to acquire samples as quickly as possible, Guava recommends using cell concentrations of at least 2.5 x 10⁵ cells/mL.
3. Mix effector and target cells at appropriate ratios (see Table 3 for suggested conditions) into a round bottom, 96-well plate.

Guava recommends setting up triplicate wells for each effector-to-target ratio used, including effectors only and targets only.

NOTE: If using purified effector cells or cell lines, Guava recommends using lower effector to target ratios, such as 20:1 or lower. If using effector cells in a heterogeneous mixture such as PBMC, use higher effector to target ratios, such

Table 3. Volumes of Target and Effector Cells Needed at the Indicated Ratios

Effector to Target Ratio	Effector Cells per well (µL)	Target Cells per well (µL)
0:1	0.00	200.00
0.2:1	33.33	166.67
0.5:1	66.67	133.33
1:1	100.00	100.00
2:1	133.33	66.67
5:1	166.67	33.33
10:1	181.81	18.19
20:1	190.50	9.50
40:1	195.12	4.88
100:1	198.02	1.98
1:0	200.00	0.00

as 100:1 to 5:1. Regardless of the effector-to-target ratio used, always set up replicate wells of effectors only and targets only.

NOTE: Incubating the cells in alternative vessels might result in a change in the amount of killing that can occur.

4. Add 200 µL of target cells only to a second set of replicate wells.
5. Cover the plates and centrifuge at 50 x g for 2 minutes.
6. Incubate plates covered at 37°C in an incubator with 5% CO₂ for 4 hours (or longer, depending on your effector cell potency).

NOTE: If longer incubation times are needed, Guava recommends incubating in 96-well, flat bottom plates.

Dead Cell Staining

1. After incubation remove plates from 37°C CO₂ incubator.
 2. Add 5 µL of Triton X-100 or NP-40 to the second set of replicate wells containing only targets only.
- IMPORTANT NOTE:** Do not allow the detergent to come into contact with any other wells as it will result in increased killing in those wells.
3. Add 40 µL of the Guava CellToxicity 7-AAD reagent, and then mix each well by repeat pipetting up and down.
 4. Incubate the samples in the dark at room temperature for 10 minutes.

Samples are now ready for acquisition on the Guava EasyCyte.

Results from NK assays using K562 target cells are stable for up to 7 hours at room temperature in the dark. Your results might vary with different effector and target cells.

7. EXPECTED RESULTS

The Guava EasyCyte CellToxicity software displays acquired data in two dot plots: a gating dot plot showing either FSC vs Green (PM3) or FSC vs Red (PM2) (Figure 1), and an analysis dot plot showing Green (PM3) vs Red (PM2).

The results are displayed on the computer screen after each sample is acquired. During acquisition, the gate in the gating dot plot acts as a counting gate. When the events within the gate reach the events to acquire, the acquisition is completed. This allows a constant number of target cells to be acquired although the effector-to-target cell ratio may vary. During analysis, events to be analyzed can be gated to remove undesired debris or dead cells using the Gating Dot Plot

by checking the **Enable Gating** box. Events above the FSC threshold falling within the rectangular gating region are displayed and analyzed on the Analysis dot plot. If **Enable Gating** is turned off (box is left unchecked), all of the events collected above the FSC threshold are displayed and analyzed on the Analysis dot plot.

The Guava EasyCyte system calculates and presents results automatically in the Analysis Results table (Figure 2).

Results include:

- % of Target Cells Killed (number of events in the upper-right quadrant divided by the number of events in the upper-right and lower-right quadrants)
- Count (number of events falling in the indicated Dot Plot region)
- Cells/mL
- % Total, or if gating is enabled, % Gated (percentage of events falling within each region)
- MFI values of each population for PM1 and PM2 (for live and dead effector and target cells)
- Gated Events (number of events that are within the gate when enabled)
- Total Count
- % Inside the Gate
- % Outside the Gate

All data and instrument settings are automatically saved in FCS 3.0 file format. You can select the option to export data to a FCS 2.0 file. This allows you to analyze Guava files with other FCS 2.0 compatible programs.

Analysis Results					
% of Target Cells Killed: 21.95 %					
	Count	Cells/mL	% Gated	PM3 MFI	PM2 MFI
Live Effector Cells	0	0.00e00	0.00	0.00	0.00
Live Target Cells	480	1.51e05	77.17	427.31	5.28
Dead Target Cells	135	4.24e04	21.70	189.61	49.55
Dead Effector Cells	7	2.20e03	1.13	28.57	43.86
All Target Cells	615	1.93e05	98.87	375.13	15.00
Gated Events	Total Count	% Inside Gate		% Outside Gate	
622	1073	57.97		42.03	

Figure 2 Analysis Results table in Guava CellToxicity software.

Guava CellToxicity assay results can vary with the cell type and assay conditions used. Figure 3 illustrates results that were obtained using the Guava EasyCyte CellToxicity Kit. In this example, effector and target cells were mixed at a 2:1 ratio with approximately 50% of the target cells killed.

8. TROUBLESHOOTING TIPS

1. Dim or false negative staining obtained with the Guava EasyCyte CellToxicity Kit may indicate reagent degradation. Verify that the reagents are not past their expiration dates before using.
2. Dim staining can indicate that the cells were not healthy enough to be properly painted. Be sure cells are in log-phase growth prior to painting.

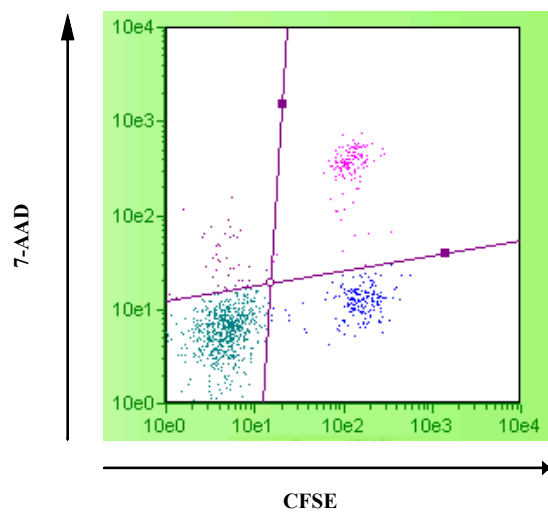


Figure 3 Detection of Live and Dead Target Cells. Unpainted, 7-AAD negative, live effector cells appear in the lower-left quadrant (teal green); painted, 7-AAD negative, live target cells appear in the lower-right quadrant (blue); unpainted, 7-AAD positive, dead effector cells appear in the upper-left quadrant (purple); and painted, 7-AAD positive, dead target cells appear in the upper-right quadrant (pink).

3. Make sure that the frozen reagents are completely thawed and well mixed before using.
4. Acquire samples as soon as possible after staining. Extended storage may adversely affect results. Avoid excessive exposure of the stained samples to light.
5. If the concentration of the stained cell sample for data acquisition is too low (<10 cells/ μ L), the accuracy of the results may be compromised. The experiment may need to be repeated at a higher cell density.
6. Be sure that samples are properly resuspended prior to acquisition. Check that the mixing option has been selected in the WorkEdit program when creating the Worklist file. Cells in the sample will settle quickly and your Guava CellToxicity results (percent of target cells killed, MFIs, and so on) will be inaccurate unless each sample is mixed prior to acquisition.
7. If the concentration of the stained cell sample for data acquisition is high (greater than 500 cells/ μ L), the Guava EasyCyte system most likely will not yield accurate results. Dilute the sample with additional PBS to bring the cell concentration into an acceptable range.
8. The default number of events to acquire is 1,000. You may enter a different number, however, your statistical error will increase as you decrease the number of events for acquisition.
9. Run Guava Check (Cat. No. 4500-0020) to verify proper instrument function and accuracy.
10. The Guava EasyCyte system performs a Quick Clean at the end of every Worklist. If your samples contain significant amounts of cellular debris that might build up in the flow system and cause a clog, you might want to select more frequent Quick Cleans from the WorkEdit program. Alternately, if your samples contain significant amounts of cellular debris, run Quick Clean with Guava ICF, followed by water, to prevent clogs or blockage.

11. If, during acquisition, the Cell Count number is not increasing and the acquisition progress bar is not moving, there is probably either insufficient volume to continue to acquire sample, or a blockage of the flow system. Check first for the lack of sufficient sample volume (on the Guava EasyCyte system, you must first pause the CellToxicity application, then eject the tray). If the sample volume is below 50 μ L, there is not enough sample for the instrument to acquire. Either add additional buffer to bring the sample volume up to greater than 50 μ L, or proceed to the next sample. If the sample volume is more than 50 μ L, then the lack of events acquired is probably due to a clog. A clog or blockage of the flow system can be caused by cell aggregates, cell debris, bleach crystals, or other particulates. Click **Pause**, then **Backflush** 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 *Guava EasyCyte System User's Guide*, or contact Technical Service for additional help.
12. For more troubleshooting tips, refer to the *Guava EasyCyte System User's Guide*, or contact Technical Support.

9. LIMITATIONS

The Guava EasyCyte system yields optimal results when the stained cell sample concentration is between 1×10^4 to 5×10^5 cells/mL. To obtain the most accurate assay results, adjust the concentration of the cell samples to within the recommended range.

10. REFERENCES

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