



Guava[®] Cytochrome c Kit



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Application

Mitochondria play a pivotal role in the generation of energy for the cell and the cascade of events associated with cell death pathways such as apoptosis. The release of key mitochondrial proteins such as Cytochrome c is an important hallmark in the pathway of apoptosis and is considered a point of no return in the apoptosis process.¹⁻⁴

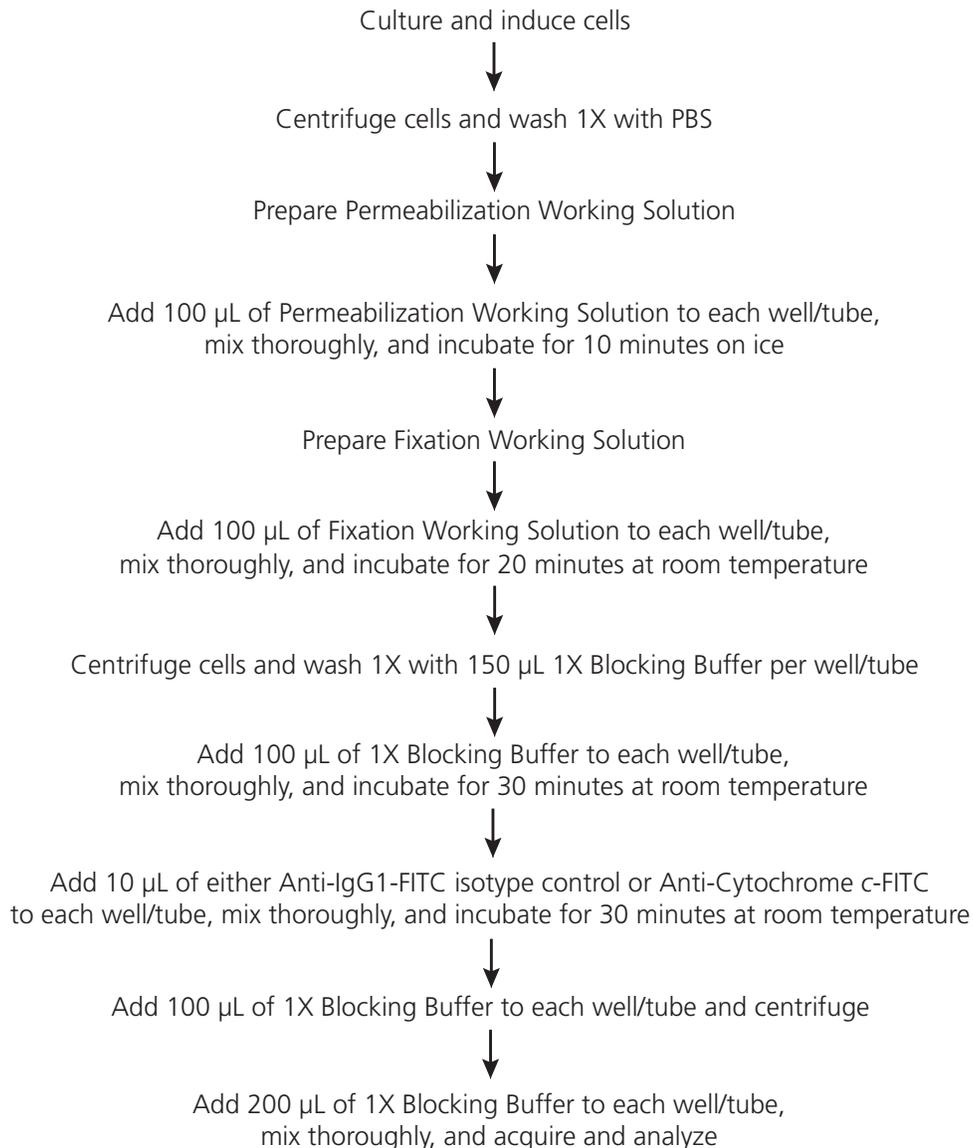
Cytochrome c is a soluble heme protein localized in the mitochondrial intermembrane space which plays an indispensable role as an electron carrier in oxidative phosphorylation during respiration, transferring electrons from the cytochrome bc1 complex to cytochrome oxidase on the surface of the inner mitochondrial membrane. On induction of apoptosis, and accompanying events such as mitochondrial depolarization, cardiolipin peroxidation,, Cytochrome c along with other pro-apoptotic proteins such as proCaspase-9, Smac/DIABLO, APF-1 and Endo F is released into the cytosol signaling the commitment to the intrinsic pathway of cell death. When released, Cytochrome c acts with other intermembrane mitochondrial proteins such as procaspase 9 and Smac, as well as cytosolic factors, including apoptotic protease activating factor-1 and ATP to activate a cascade of caspases that promote cell death. The quantification of the release of Cytochrome c from the mitochondria of apoptotic cells can be used to characterize the mitochondrial-dependent pathway to cell death.⁵⁻¹¹

Current techniques for evaluation of release of Cytochrome c include methods such as Western blots, ELISA and fluorescence microscopy, which are time consuming and laborious and make it cumbersome to assess multiple samples in parallel. Further, since methods like Western blots or ELISA typically involve lysis of cells, the Cytochrome c translocation information on a per cell basis is lost. Further, in methods involving fractionation and isolation of mitochondrial proteins which often employ harsh mechanical means such as homogenization, there may be incomplete or excessive rupture of mitochondria which can cause the amount of Cytochrome c to be over or under-estimated.

The Guava® Cytochrome c kit provides for a simpler, gentler and a faster method to assess levels of Cytochrome c in mitochondria of cells undergoing apoptosis on a per cell basis using flow cytometry. The kit provides a means of identifying apoptotic cells and provides as a method for inhibitor /compound screening and assessing the regulation of apoptotic signaling in cells. In combination with analysis by flow cytometry the Cytochrome c kit provides researchers an important tool in assessing regulation of apoptotic signaling in cells.

Test Principle

The Guava® Cytochrome c Kit assesses the loss of mitochondrial Cytochrome c in cells that have been induced to undergo apoptosis. The kit includes a directly labeled Anti-Cytochrome c-FITC Antibody, Anti-IgG₁-FITC Isotype control, along with optimized fixation, permeabilization, and blocking buffers to allow the detection of Cytochrome c using flow cytometry. The assay kit may be used on a flow cytometer equipped with a 488-nm laser which allows the detection of FITC probes. The kit uses specialized buffer sets to achieve selective permeabilization of mitochondria while leaving the mitochondrial membrane intact. Viable or live cells will demonstrate higher levels of Cytochrome c staining while apoptotic cells which have released their Cytochrome c from the mitochondria to the cytoplasm will demonstrate reduced staining intensity when probed with an anti-Cytochrome c FITC antibody. Stained cells can then be analyzed by flow cytometry. The entire staining can be performed in 2 hours once cells are ready as shown in the flow chart in Figure 1. Sufficient reagents are provided for 100 samples. The kit includes all optimized fluorescently labeled antibodies and buffers necessary for cell preparation and analysis. The flow cytometry method described here can be stained and analyzed within 3 hours of completion of induction for as many as 96 samples.

Figure 1: Guava[®] Cytochrome c Kit Workflow

Kit Components

- Anti-Cytochrome c- FITC Antibody (Part No. 4700-1320); One vial containing 1 mL of antibody
- Anti-IgG₁-FITC isotype Control (Part No. 4700-1340); One vial containing 100 µL of antibody
- 10X Permeabilization Buffer (Part No. 4200-0636); One vial containing 1 mL buffer
- 5X Fixation Buffer (Part No. 4300-0340); Two vials containing 3 mL buffer
- 1X Blocking buffer (Part No. 4200-0635); One bottle containing 55 mL buffer

Materials Not Supplied

1. Guava® System (EasyCyte™, EasyCyte™ Mini, or easyCyte HT System) with Guava CytoSoft™ or GuavaSoft Software containing the ExpressPro or InCyte™ Software module or equivalent flow cytometry system with ability to detect FITC.
2. ViaCount™ reagent (Catalog No. 4000-0041) or ViaCount Flex reagent (Catalog No. 4700-0060)
3. Cell line of interest
4. Media for cell line of interest
5. Tissue culture instruments and supplies (including 37°C incubator, growth media, plates, detachment buffer, etc.)
6. Polypropylene tubes and or bottles for sample and buffer preparation and storage.
7. 96-well plates, if desired for sample preparation.
8. Pipettors with corresponding tips capable of accurately measuring 1-1000 µL
9. Tabletop centrifuge capable of exceeding 300 xg.
10. Vortex mixer
11. 1X Phosphate buffered saline (PBS) without calcium or magnesium.
12. Reagent reservoirs, optional
13. Guava Instrument Cleaning Fluid (ICF) (Catalog No. 4200-0140), optional
14. Guava easyCheck™ Kit (Catalog No. 4500-0025), optional
15. 20% bleach solution

Precautions

- Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this product.
- The instructions provided have been designed to optimize the kit's performance. Deviation from the kit's instructions may result in suboptimal performance and may produce inaccurate data.
- Some assay components included in the kit may be harmful. The kit includes a formaldehyde solution (fixation solution). Please refer to the SDS for specific information on hazardous materials. Safety Data Sheets for our kits are available from our web site (www.luminexcorp.com) or by contacting Technical Support.
- All fluorochrome conjugated antibodies are light sensitive and must be stored in the dark at 4°C.
- During storage and shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For maximum recovery of product, centrifuge vial briefly prior to removing cap.
- The Guava® Cytochrome c Kit contains sodium azide, which is toxic.
- Avoid microbial contamination of the solution, which may cause erroneous results.

Storage

Upon receipt, store the kit as follows:

- 20°C desiccated
 - 10X Permeabilization Buffer



CAUTION: Permeabilization Buffer is highly hygroscopic avoid multiple freeze thaws and needs to be stored desiccated. Aliquot 10X Permeabilization Buffer into appropriate test sizes.

- 2-8°C
 - Anti-Cytochrome c- FITC Antibody, Anti-IgG₁-FITC isotype control, 1X Blocking Buffer .



CAUTION: Fluorochrome-conjugated antibodies should always be stored at 4°C. Any deviation in temperature for long periods of time may compromise the performance of the antibodies.

- Room temperature
 - 5X Fixation Buffer

Preparation of Buffers

Prepare each of these buffers fresh each day of your experiment. Each buffer is only stable for 4 hours at room temperature. To ensure there is enough buffer for your samples mix n+2 samples, where n is the number of tests you wish to run for each experiment, plus one for the isotype control, plus one extra.

- Permeabilization Buffer Working Solution:** Mix the following reagents together. Make sure to add the 5X Fixative Solution first, followed by 1X PBS without calcium and magnesium, and the 10X Permeabilization Buffer last.

Component	1 Test	25 Tests	100 Tests
5X Fixation Solution	2.5 µL	62.5 µL	0.25 mL
1X PBS	87.5 µL	2187.5 µL	8.75 mL
10X Permeabilization Buffer	10 µL	250 µL	1 mL
Total Volume	100 µL	2500 µL	10 mL

NOTE: Make sure that 10X Permeabilization buffer is completely thawed before mixing. Pipette material up and down to ensure proper mixing.

2. **Fixation Buffer Working Solution:** Mix the following reagents together.

Component	1 Test	25 Tests	100 Tests
5X Fixation Solution	40 µL	1000 µL	4 mL
1X PBS	60 µL	1500 µL	6 mL
Total Volume	100 µL	2500 µL	10 mL

Before You Begin

This protocol was developed to allow direct determination of the percent of cells that have released their Cytochrome c from the mitochondria into the cytoplasm. For optimal throughput during acquisition, final cell concentrations should be between 2×10^4 and 1×10^5 cells/well (or 1×10^5 to 5×10^5 cells/mL), although apoptosis can be detected in cultures with as few as 2×10^3 cells/well (or 1×10^4 cells/mL). Care should be taken to keep cell concentrations as constant as possible in all samples of an experiment. The mean fluorescent intensity of Cytochrome c-FITC bound to cells can vary significantly with a two-fold change in cell concentration, although the percentage of cells bound by

Cytochrome c-FITC remains constant. However, if the cell concentration exceeds 5×10^5 cells/mL, the Cytochrome c-FITC reagent may be in limiting concentration and will therefore bind to fewer cells, resulting in lower percentages for Cytochrome c-FITC negative cells.

Cells should be acquired shortly after the sample preparation had been completed. While cells are fixed, and some cell lines have been shown to yield stable results for up to 24 hours, post preparation stability may be different for your particular cell line.

The following procedures for cell staining are guidelines. Different cell types have varying Cytochrome c within their mitochondria. Different cell lines may have more optimal permeabilization and staining conditions. You may need to adjust the amount of reagents used for optimal staining of your cell samples. If this is the case, please follow the recommendations described in "Cell Staining Procedure" on page 6.

Time considerations: The process of staining cells with the Guava[®] Cytochrome c Kit takes approximately 2 hours. Acquiring data on your Guava System usually takes approximately 1 hour but can vary depending on your cell concentration. However, preparing cells for testing requires periodic maintenance and cultivation several days in advance. Once you cultivate the proper number of cells for your experiment, it can take an additional 2 to 48 hours of culture with various inducers to stimulate detectable apoptosis.

Make sure to include proper controls for instrument set up and accurate results. Luminex recommends including an isotype control for both your positive and negative controls.

NOTE: For details on how to culture and prepare cell samples, including positive and negative control samples, for the Guava Cytochrome c Kit, see "Appendix A: Cell Sample Preparation" on page 12.

Cell Staining Procedure

1. Allow Guava[®] Cytochrome c Reagents warm to room temperature.
2. Prepare required buffers as described under "Preparation of Buffers" on page 4.
3. Prepare samples for testing. For instructions on making cell suspensions, see "Appendix A: Cell Sample Preparation" on page 12.
4. Add 1.5×10^4 to 1.5×10^5 cells in 200 μL (7.5×10^4 to 7.5×10^5 cells/mL) of cells in suspension to each well or tube.
5. Centrifuge cells at 300 xg for 5 to 7 minutes.
6. Aspirate off the supernatant and add 200 μL of 1X PBS to each well or tube. Mix each sample thoroughly.
7. Centrifuge cells at 300 xg for 5 to 7 minutes and aspirate off supernatant.
8. Add 100 μL of Permeabilization Buffer Working Solution to each well or tube and mix thoroughly.
9. Cover the plate with a plate sealer or cap each tube and incubate for 10 minutes on ice.
10. After permeabilization incubation add 100 μL of the Fixation buffer Working Solution prepared to each sample and mix each sample thoroughly.
11. Incubate at room temperature for 20 minutes.
12. After incubation, centrifuge the cells for 5 to 7 minutes at 300 xg.
13. Aspirate off the supernatant and add 150 μL of 1X Blocking Buffer to each sample.
14. Centrifuge cells for at least 5 to 7 minutes at 300 xg.
15. Aspirate off the supernatant and add 100 μL of 1X Blocking Buffer to each sample and mix thoroughly.
16. Incubate the plate for 30 minutes at room temperature.
17. After incubation add 10 μL of either the Anti-IgG₁-FITC Isotype Control or Anti-Cytochrome c-FITC Antibody to each sample and mix thoroughly.
18. Incubate the plate at room temperature for 30 minutes in the dark.
19. After incubation, add 100 μL of 1X Blocking Buffer to each sample.
20. Centrifuge sample for at 5 to 7 minutes at 300 xg.
21. Aspirate off the supernatant, and add 200 μL of 1X Blocking Buffer to each well.
22. Samples are now ready for acquisition on a flow cytometer.
23. Read within 4 hours.

Sample Data

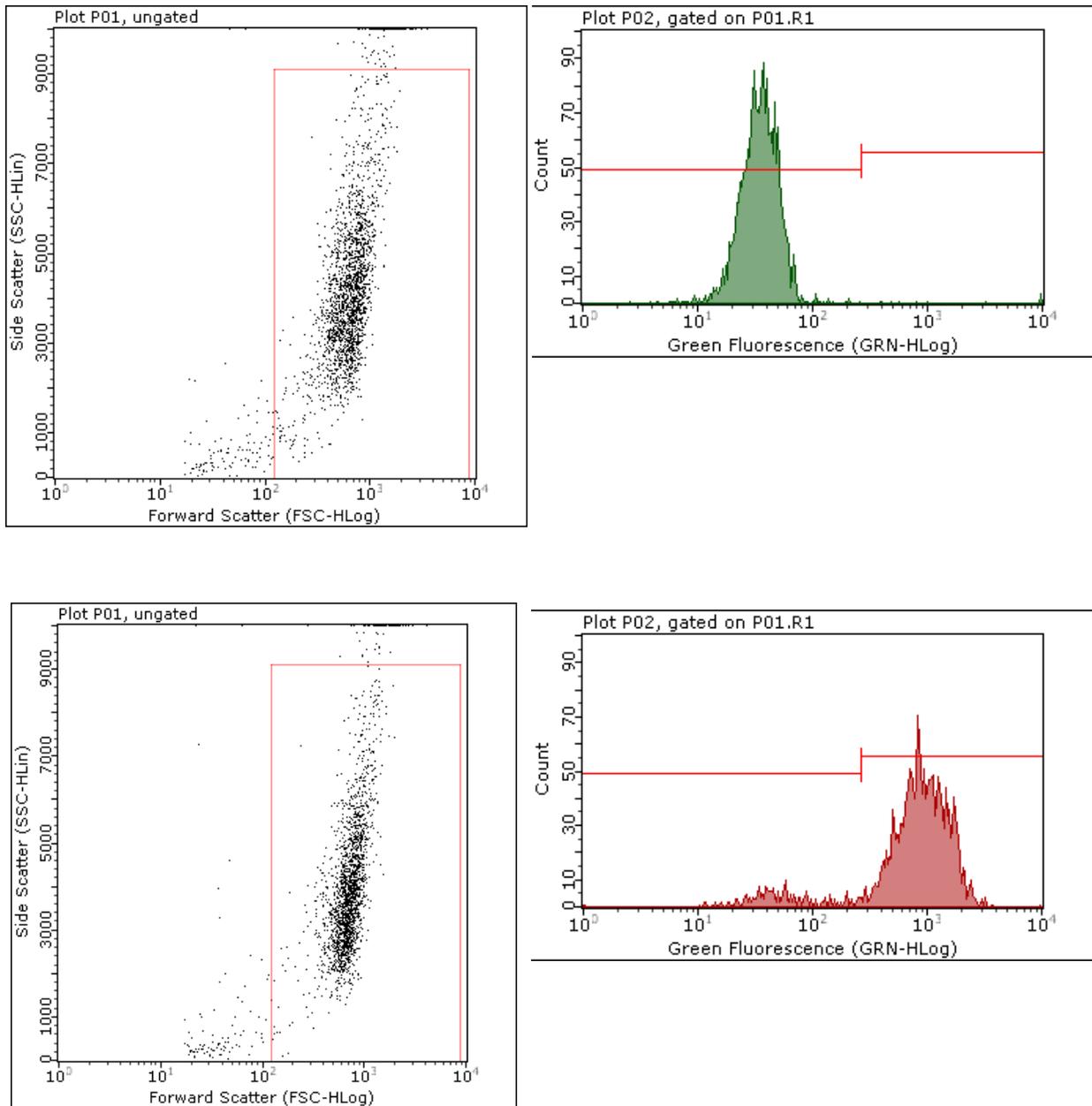


Figure 2: Display of Plots for Sample Acquisition: Set up of plots for data acquisition for samples treated with the Cytochrome c Kit. Two plots are typically set up for data acquisition. The first dot plot on the left, FSC (log) vs. SSC is typically used as a counting gate to eliminate debris. The second plot shown on the right is a histogram of the green (FITC) channel. Panel A shows cells stained with the Isotype Control. Cells stained with the Isotype control show a low green fluorescence. Center your cells on the histogram so it is between 10¹ and 10². Plots in Panel B show live cells (negative control) stained with Cytochrome c-FITC. Cells stained with Cytochrome c-FITC show a high fluorescence. Ensure permeabilization and staining of the cells has occurred properly by checking that there is an upward shift when compared to the isotype control. Set one marker to include live or uninduced cells and a second marker to include apoptotic or dead cells, and the isotype control.

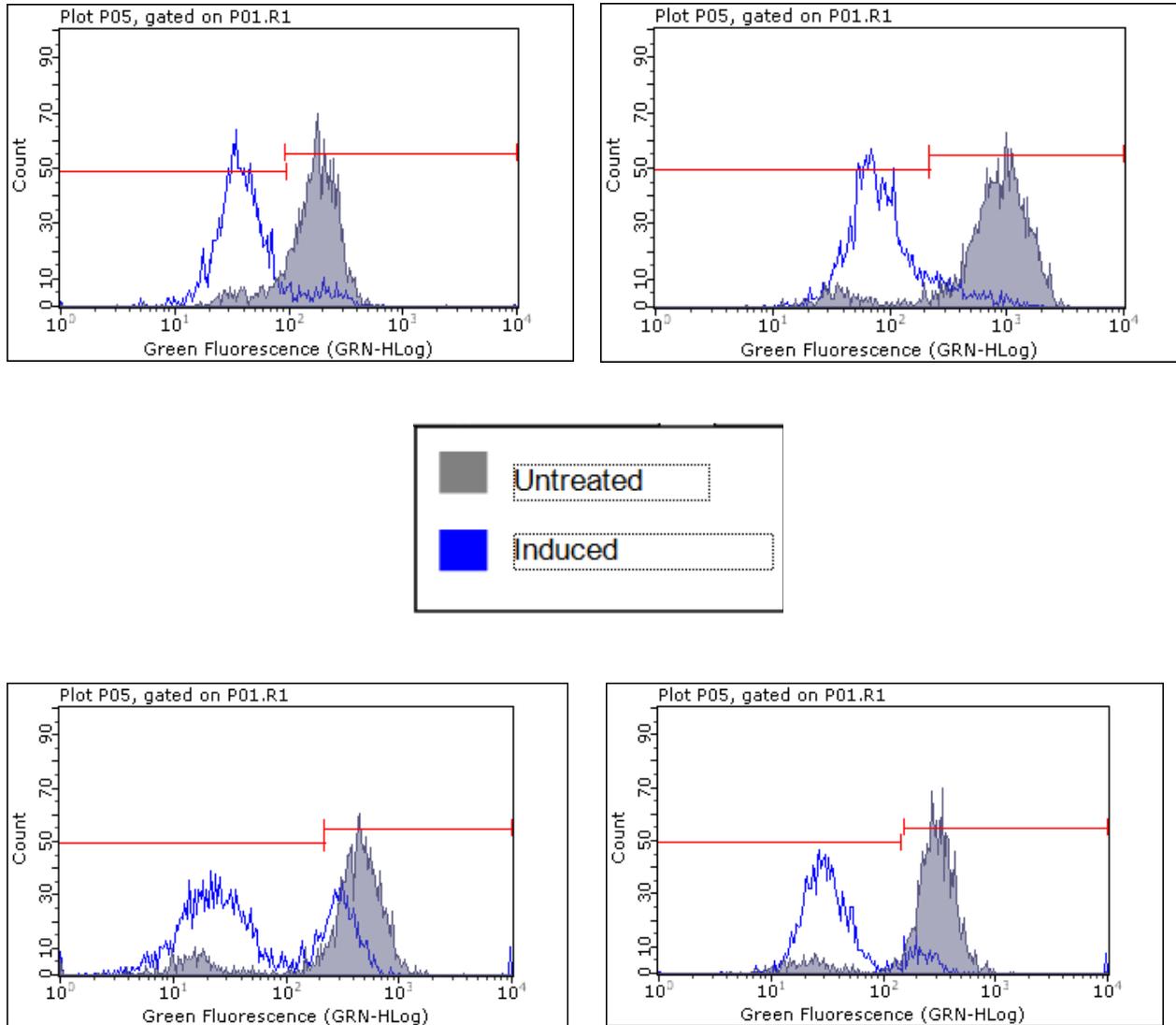


Figure 3: Histograms depicting cells stained using the Guava[®] Cytochrome c Kit. Plot A shows Jurkat cells untreated or induced with 1 μM staurosporine for 2 hours. Plot B shows HeLa cells untreated or induced with 3 μM staurosporine for 4 hours. Plot C shows HI-60 cells untreated or induced with 33 μM anisomycin for 4 hours. Plot D shows HB cells untreated or induced with 100 μM for 4 hours. Plots show downward shifts in fluorescence of levels of Cytochrome c for multiple cell lines and inducers for the Guava Cytochrome c Kit.

Technical Hints

- If minor precipitate is detected in the 10X Permeabilization Buffer, place the tube in a 56°C water bath for 30 minutes, followed by mixing the contents on a mechanical vortex. Before adding solution to cells allow cooling to room temperature for 1 hour.
- For cellular staining and analysis to be most effective, make sure that test cells have good viability prior to use.
- For certain cell cultures cell pellets may become hazy or transparent following the fixation step, making it difficult to see. If sampling a small collection of cells for flow analysis, it is recommended that after each centrifugation step, 20 μ L is left behind.
- To ensure optimal staining, avoid mixing of multiple kit lots.

Troubleshooting

Potential Problem	Experimental Suggestions
Acquisition rate decreases dramatically Instrument clogging Too many cells	<ul style="list-style-type: none"> Cell concentration too high - Decrease the number of cells per microliter by diluting sample to 300–500 cells/μL. The Guava® EasyCyte™ Plus or Guava easyCyte HT system gives the most accurate data when the flow rate is less 500 cells/μL. Run a Clean and Rinse to clean out the capillary. This procedure can be performed during or after an assay. This will wash away any material forming within the glass capillary walls.
To few cells	Spin down cells and resuspend in a smaller volume. The assay instructions are optimized to give you a range of cells between 100–500 cells/ μL in the final sample volume. However, cell loss is common during washing steps in the assay procedure. A substantial decrease in cell numbers can lead to difficulty in adjusting settings. Make sure to leave the cell pellet intact when discarding buffer. If the cells are not generating a compact pellet after centrifugation, increase the time to 7 minutes and/or increase the speed by 300 xg until a compact and visible cell pellet forms.
Background and/or non-specific staining of cells	Although the assay procedure has been optimized to function utilizing many different cell types, further antibody titrations may be necessary for some cell types to capture the ideal staining concentration. Non-specific staining and background may indicate that less antibody will need to be used during the staining procedure.
High background staining for adherent cells	The cells may be damaged. Avoid damaging adherent cells when removing them from their substrate.
Staining is weak	<ul style="list-style-type: none"> Cells may not be fully induced. To ensure proper induction conduct a time-course or concentration study in order to achieve the best results for Cytochrome c loss. Some cells may require a longer permeabilization time. Optimization of permeabilization time may need to be optimized.
Uninduced Cells show now upward shift when compared to the isotype control	If live or negative control cells show no upward shift when compared to the isotype control, permeabilization may not have occurred correctly. Ensure complete permeabilization by making sure no precipitate is present when diluting the 10X permeabilization solution, mixing the Permeabilization working solution is prepared in the correct order, and there is thorough mixing when adding the permeabilization working solution.
No downward shift	<ul style="list-style-type: none"> Live cells may have been permeabilized for too long, ensure that 10 minutes is the maximum permeabilization as to not kill the uninduced cells. Cells may not have been induced. Positive controls should be included for each experiment to ensure accurate staining protocol. Treatments to induce apoptosis in various cell lines include, but are not limited to a) serum starvation, b) activation of cell surface receptors such as Fas, TNFR1, or TCR, c) UV irradiation, and d) treatment with a compound that is known to induce apoptosis in your sample.

Potential Problem	Experimental Suggestions
Samples appear to be induced when low level of induction is expected	Cell cultures may be compromised. Negative controls should be a sample from your cell culture, not treated to induce apoptosis. Negative control samples show low levels of Cytochrome c loss. Sub-optimal culture conditions may stress cells in culture, causing them to undergo apoptosis in the absence of experimental induction treatment. The negative control from a stressed culture often shows a downward shift in Cytochrome c.

Contact Luminex Technical Support for additional assistance.

References

1. Newmeyer DD and Ferguson-Miller S Mitochondria: releasing power for life and unleashing the machineries of death. *Cell*. 2003;112:481-490
2. Campos C, Paim B, Cosso R, Castilho R, Rottenberg H, Vercesi A. Method for Monitoring of Mitochondrial Cytochrome c release during cell death: Immunodetection of Cytochrome c by Flow Cytometry after selective permeabilization of the plasma membrane. *Cytometry*. 2006;69A:515-523
3. Finkel E. The mitochondrion: is it central to apoptosis? *Science*. 2001;292:624-626.
4. Zamzami N, Kroemer G. The mitochondrion in apoptosis: how Pandora's box opens. *Nat Rev Mol Cell Biol*. 2001;2:67-71.
5. King M, Eddaoudi A, Davies D. A Comparison of Three Flow Cytometry Methods for Evaluating Mitochondrial Damage During Staurosporine-Induced Apoptosis in Jurkat Cells. *Cytometry*. 2007;A:668-674.
6. Yang J, Liu X, Bhalla K, Kim CN, Ibrado AM, Cai J, Peng TI, Jones DP, Wang X. Prevention of apoptosis by Bcl-2: Release of Cytochrome c from mitochondria locked. *Science*. 1997;275:1129-1132. CAMPOS ET AL.
7. Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD. The release of Cytochrome c from mitochondria: A primary site for Bcl-2 regulation of apoptosis. *Science*. 1997;275:1132-1136.
8. Khosravi-Far R, Esposti MD. Death receptor signals to mitochondria. *Cancer Biol Ther*. 2004;3:1051-1057.
9. Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. *Science*. 2004;305:626-629.
10. Jiang X, Wang X. Cytochrome c-mediated apoptosis. *Annu Rev Biochem*. 2004;73:87-106.
11. Marchetti P, Castedo M, Susin SA, Zamzami N, Hirsch T, Macho A, Haeffner A, Hirsch F, Geuskens M, Kroemer G. Mitochondrial permeability transition is a central coordinating event of apoptosis. *J Exp Med*. 1996;184:1155-1160.

Related Kits

1. Guava[®] MitoPotential Red Kit (Catalog No. FCCH100105)
2. Guava[®] MitoDamage Kit (Catalog No. FCCH100106)
3. Guava[®] MitoLive Kit (Catalog No. FCCH100107)
4. Guava[®] Annexin Red Kit (Catalog No. FCCH100108)
5. Guava[®] MitoStress Kit (Catalog No. FCCH100109)
6. Guava[®] EasyCyte MitoPotential Kit (Catalog No. 4500-0250)
7. Guava Nexin[®] Reagent (Catalog No. 4500-0450, 4500-0455)
8. Guava Caspase Kits (Catalog Nos. 4500-0500 to 4500-0650)

Appendix A: Cell Sample Preparation

Preparing Controls

Regardless of the type of cells (adherent or non-adherent) 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 undergo apoptosis. The stained negative control sample should be run at the beginning of the experiment, and used to adjust the instrument settings for baseline fluorescence.
- Positive control sample: The positive control should be a sample of apoptotic and dead cells from your cell culture treated with 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, as well as non-adherent and adherent cells cultured in flasks or in other tissue culture vessels. Each culture condition requires different protocols to harvest the cells. Thereafter, the staining protocols are identical.

Preparing non-adherent cells cultured in 96-well plates

1. Set up initial culture conditions, such that after culture and treatment, cells are at concentration of 2×10^4 to 1.5×10^5 cells in 200 μ L of serum- or albumin containing medium (2×10^5 to 7.5×10^5 cells/mL).
2. Proceed to "Cell Staining Procedure" on page 6.

Preparing adherent cells cultured in 96-well plates

For harvesting adherent cells, we suggest using Guava ViaCount Cell Dispersal Reagent (Catalog No. 4700-0050) instead of other enzymatic treatment. ViaCount Cell Dispersal Reagent contains proteases that cleave proteins, nucleases that cleave DNA and RNA, and collagenases that cleave collagen, among other enzymes. Hence, ViaCount Cell Dispersal Reagent is preferred for detaching adherent cells and dispersing cell clumps over other

enzymatic treatments, such as trypsin. Alternately, some cells can be dislodged mechanically or by using an EDTA solution.

1. Set up initial culture conditions, such that after culture and treatment, cells are at concentration of 2×10^4 to 1.5×10^5 cells in 100 μL (2×10^5 to 7.5×10^5 cells/mL).
2. Centrifuge cells at 300 xg for 5 to 7 minutes.
Adherent cells may detach from the plate as they undergo into apoptosis. Spinning the cells down prior to detaching ensures that all cells are at the bottom of the well before aspirating.
3. Aspirate off the culture medium and rinse each well once with 50 μL of PBS.
4. Pipette the PBS wash, which may contain detached apoptotic cells, into a fresh 96-well plate.
5. Dilute the ViaCount Cell Dispersal Reagent 1:3 with PBS.
6. Add 50 μL of diluted ViaCount Cell Dispersal Reagent to each well and incubate at 37°C for 3 to 5 minutes (or until cells begin to detach).
7. Add 100 μL of medium (containing at least 5% BSA or serum) to each well and pipette repeatedly to detach cells from the well bottoms.
8. Add the 50 μL of PBS from step 5 back into each well.
9. Centrifuge cells at 300 xg for 5 to 7 minutes.
10. Aspirate the culture medium, being careful not to disturb the cell pellet.
11. Add 200 μL of fresh serum- or albumin-containing medium to each well.
12. Proceed to "Cell Staining Procedure" on page 6.

Preparing non-adherent cells cultured in flasks or other tissue culture vessels

1. Transfer between 2×10^4 and 1.5×10^5 cells in 200 μL of serum- or albumin-containing medium (2×10^5 to 7.5×10^5 cells/mL) to each well in a 96-well round bottom plate or into a 1.5-mL microcentrifuge tube.
2. Proceed to "Cell Staining Procedure" on page 6.

Preparing adherent cells cultured in flasks or other tissue culture vessels.

For harvesting adherent cells, we suggest using Guava ViaCount Cell Dispersal Reagent (Catalog No. 4700-0050) instead of other enzymatic treatments. ViaCount Cell Dispersal Reagent contains proteases that cleave proteins, nucleases that cleave DNA and RNA, and collagenases that cleave collagen, among other enzymes. Hence, ViaCount Cell Dispersal Reagent is preferred for detaching adherent cell and dispersing cell clumps over other enzymatic treatments, such as trypsin. Alternately, some cells can be dislodged mechanically or by using an EDTA solution.

1. Remove culture medium from flask and place in a 50-mL conical screw cap tube.
NOTE: This is to retain any detached apoptotic or dead cells present in the flask.
2. Wash cells with 10 mL of 1X PBS.
3. Remove PBS and place in 50-mL conical screw cap tube used in step 1.
4. Dilute the ViaCount Cell Dispersal Reagent 1:3 with PBS.
5. For a T-75cm² flask, add 3 mL of diluted ViaCount Cell Dispersal Reagent and incubate at 37°C for 3 to 5 minutes (or until cells begin to detach).
6. Add 6 mL of medium with serum and pipette repeatedly to release cells from the bottom of the flask.

NOTE: If the cells are typically grown in serum- or protein-free medium, then serum or BSA must be added to the medium to a final concentration of at least 5%. The addition of the protein is necessary to neutralize the activity of the enzymes in the ViaCount Cell Dispersal Reagent.

7. Transfer released cells to the 50-mL conical screw cap tube used in step 1.
8. Centrifuge cells at 300 xg for 5 to 7 minutes.
9. Aspirate the culture medium, being careful not to disturb the cell pellet.
10. Add fresh serum- or albumin-containing medium to the 50-mL tube.
11. Count cells in suspension using either Guava ViaCount Reagent or Guava ViaCount Flex Reagent and adjust the cell concentration, if necessary.
12. Transfer 2×10^4 to 1.5×10^5 cells in 100 μ L of serum- or albumin-containing medium (2×10^5 to 7.5×10^5 cells/mL) to each well in a 96-well microplate or into a 1.5-mL microcentrifuge tube.
13. Proceed to "Cell Staining Procedure" on page 6.

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