



## Guava® EasyCyte Caspase 3/7 Kit

Cat. No. 4500-0290  
100 tests

**For Discriminating Apoptotic from Non-Apoptotic Cells  
on the Guava EasyCyte System Based on the  
Detection of Caspase 3/7 Enzyme Activity**

**Research Use Only  
Not for Use in Diagnostic Procedures**

### 1. PRODUCT DESCRIPTION AND INTENDED USE

Caspases (cysteinyI-directed aspartate-specific proteases)<sup>1-3</sup> are central components in propagating the process of programmed cell death (apoptosis) in response to pro-apoptotic signals. Inactive in healthy cells, members of the caspase family not only act to initiate the intracellular event cascade (Caspase-2, -8, -9, -10) but also operate further downstream directing cellular breakdown through cleavage of structural proteins (Caspase-3, -6, -7) and cytokine processing (Caspase-1, -4, -5, -11 to -14). Identification of the active caspases indicates how far cells have progressed in the apoptotic program.

The Guava EasyCyte Caspase 3/7 Kit simultaneously determines both a live cell count and the relative percentage of cells in the various stages of apoptosis. The kit employs a Fluorescent Labeled Inhibitor of Caspases (FLICA™) technology that will specifically identify the presence of active Caspase-3 and -7 molecules. The FLICA reagent is comprised of 3 subunits – an amino acid inhibitor sequence recognized by the caspase 3/7 aspartic acid-glutamic acid-valine-aspartic acid (DEVD); a fluoromethylketone moiety (FMK) which covalently binds active caspase 3/7 enzymes; and a carboxyfluorescein (FAM) reporter. Cell permeant and non-cytotoxic, the FLICA reagent covalently binds to active caspase 3/7 and is retained in the cell, while unbound FLICA molecules diffuse out of the cell and are washed away. Cells showing significant positive FLICA staining correspond to apoptotic cells. The cell impermeant dye, propidium iodide (PI), is included in the assay to permit simultaneous evaluation of caspase activity and membrane integrity. PI is excluded from live (healthy) and early apoptotic cells, but permeates later stage apoptotic and dead cells.

### 2. ASSAY OVERVIEW

**IMPORTANT:** Please read this entire insert before performing this assay.

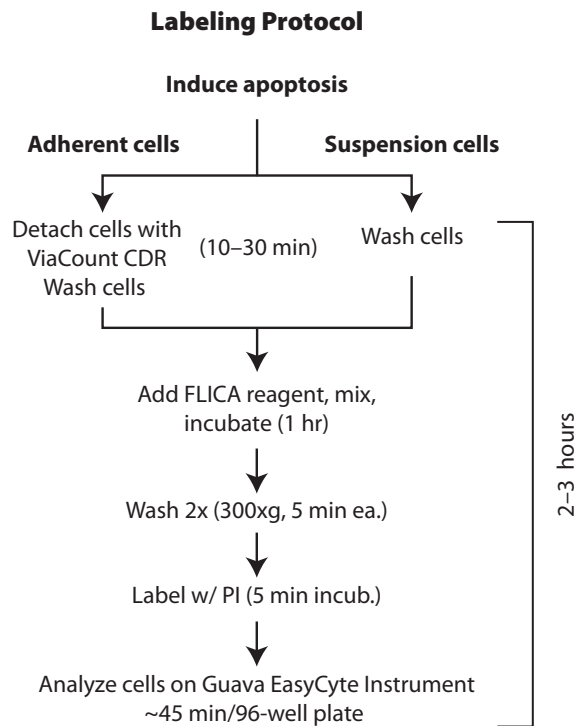


Figure 1. Overview of Guava Caspase 3/7 Assay.

**Recommended assay conditions.** The Guava EasyCyte Caspase 3/7 Detection Kit has been specially optimized for a 96-well microplate assay format. A typical assay test (single well of a 96-well microtiter plate) uses 10 µL of 10X FLICA reagent and 0.75 µL PI to stain  $5 \times 10^4$  cells in a reaction volume of 100 µL. Following these specifications there are 100 tests/kit or one 96-well plate per kit. We recommend inclusion of the following control samples in your assay:

- Negative control for apoptosis (stained, non-induced cells)
- Positive control for apoptosis (cells treated with a proven apoptotic inducer and stained)

To insure proper color compensation for data acquisition, we also recommend two additional samples:

- Positive control for apoptosis stained only with FLICA
- Positive control for apoptosis stained only with PI

**Time Considerations.** Staining and data acquisition on the Guava EasyCyte system for one 96-well microplate can usually be completed in 3-4 hours. However, the assay uses living cells that require periodic maintenance and cultivation several days in advance. Once the proper number of cells for your experiment has been cultivated, additional time is required for the apoptosis induction process (typically requires 2-4 more hours depending on your cell type and method). Prepare the kit reagents near the end of the induction period so they can be used once the cells are ready for staining.

### 3. MATERIALS PROVIDED

Guava EasyCyte Caspase 3/7 Detection Kit (Catalog No. 4500-0290, 100 tests/kit) includes the following reagents:

- FAM-DEVD-FMK Caspase 3/7 reagent (Part No. 4700-0250, lyophilized FLICA reagent, 100 tests)
- Propidium Iodide (Part No. 4700-0270, 1 mL @ 250 µg/mL in water)
- Anhydrous DMSO (Part No. 4300-0160, 500 µL)
- Wash Buffer (10x), (Part No. 4200-0163, 15 mL)

### 4. HANDLING AND STORAGE

1. Store unopened reagent vials at 2–8°C. Unopened reagents are stable for up to 1 year.
2. Protect FLICA reagent from light exposure.
3. Once reconstituted, 250X FLICA reagent stock solution may be diluted for immediate use or stored at < -20°C where it is stable for up to 6 months from the date of reconstitution. The reagent should not be thawed more than twice during that time. Repeated cycles of freezing and thawing will diminish reagent activity. Protect from light during handling. Diluted 1X reagent must be used immediately.
4. Once diluted, store 1X Wash Buffer at 2–8°C for up to 14 days.

### 5. WARNINGS AND PRECAUTIONS

1. The Guava EasyCyte Caspase 3/7 Kit is intended for research use only.
2. Exercise care to avoid cross contamination of samples during all steps of this procedure as this may lead to erroneous results.
3. Use gloves while handling FLICA reagent, PI, and DMSO. Avoid contact with skin and mucous membranes.
4. Dispose of all materials using proper precautions in accordance with federal, state, and local regulations.
5. Material Safety Data Sheets (MSDS) for kit reagents are available from our web site ([www.guavatechnologies.com](http://www.guavatechnologies.com)) or by contacting Guava Technical Support ([support@guavatechnologies.com](mailto:support@guavatechnologies.com)).
6. Guava does not warrant using the reagents after the expiration date.
7. The 10X Wash Buffer contains sodium azide as a preservative. Sodium azide 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. EQUIPMENT AND MATERIALS REQUIRED

### Equipment:

- Guava EasyCyte with CytoSoft™ software
- Vortex mixer
- Centrifuge with 96-well microplate holders
- 37°C CO<sub>2</sub> incubator
- Serological pipets, sterile
- Micropipettors
- Multipipettors, 2–200 µL capacity
- 8-channel microplate aspirator (VWR Cat. No. 29443-120 or equivalent)
- Disposable micropipettor tips
- V-bottom reagent reservoirs, 55 mL (VWR Cat. No. 210070-970 or equivalent)
- 1.5 mL microfuge tubes (VWR Cat. No. 20170-215) for cleaning

### Reagents/Materials For Cell Culture and Staining (based on 96-well plate format):

- Cell culture medium appropriate for cell lines used
- 1X Phosphate buffered saline (PBS) or equivalent, pH 7.2 to 7.4
- Cultured cells, primary cells or cell lines
- 96-well microplates, round bottom (Falcon Cat. Nos. 353910 or 353918 or equivalent). Refer to *Guava EasyCyte System User's Guide* for other compatible microplates.
- 96-well microplates, flat bottom (Falcon Cat. Nos. 353075 or 353915) or hydrogel-coated flat bottom (Costar Cat. No. 3474, Myriad Industries Cat. No. 0400-3474)
- ViaCount CDR (Cell Dispersal Reagent) for adherent cells (Cat. No. 4700-0050), optional
- Guava ViaCount® Reagent (Cat. No. 4000-0040), optional
- Caspase kit (For contents see Section 3)

### Reagents for Instrument Maintenance:

- Guava Check beads (Cat. No. 4500-0020)
- Deionized water
- 20% bleach solution
- Guava ICF™ Instrument Cleaning Fluid (Cat No. 4200-0140)

## 6. REAGENT AND SAMPLE PREPARATION

### Reagent Preparation

- **1X Wash Buffer.** The wash buffer is supplied as a 10X concentrate, which must be diluted to 1X with deionized water prior to use (mix 1 part Wash Buffer (10X) with 9 parts deionized water). If necessary, gently warm (no more than 37°C) Wash Buffer (10X) to completely dissolve any salt crystals that may have precipitated out of solution during storage. Store 1X Wash Buffer at 2–8°C for up to 14 days. Approximately 3.5 mL of 10X Buffer is required per 96-well stained.
- **250X FLICA stock solution.** The FLICA reagent is supplied as a highly concentrated lyophilized powder. It must first be reconstituted with DMSO to make a 250X stock concentrate.
  1. Reconstitute each vial of lyophilized FLICA with 40 µL DMSO. This yields a 250X stock solution.

2. Mix by swirling and tilting the vial until completely dissolved. At room temperature the reagent should be completely dissolved within a few minutes.
3. If using this solution immediately dilute it to 10X (see below).
4. If using later, prepare small aliquots of the 250X FLICA stock and store unused at ≤ -20°C, protected from light.

**Note:** 40 µL of 250X FLICA solution is enough to perform 100 tests.

- **10X FLICA working solution.** Using the freshly reconstituted or thawed 250X FLICA stock, prepare a 10X working solution by diluting the stock 1:25 with PBS (for example, 40 µL of 250X stock FLICA and 960 µL PBS yields 1000 µL of 10X FLICA working solution). Using this suggested protocol, each sample to be tested requires 10 µL of 10X FLICA. The 10X FLICA working solution must be used the same day that it is prepared.
- **Propidium Iodide.** This reagent is ready to use without further preparation.

### Cell Sample Preparation for Cells Cultured in Microtiter Plates

1. Culture cells in 96-well microplates to density optimal for apoptosis induction according to your specific protocol. Do not exceed  $5 \times 10^5$  cells/mL in a final volume of 100 µL ( $5 \times 10^4$  cells/well) in the final sample.
2. Induce apoptosis in each test well using the desired protocol.
3. For positive control samples, treat some of the cells in the 96-well microplates with a proven apoptosis induction method. These cells will not only serve as an internal control for apoptosis but will also be used for single color compensation of the FLICA and PI fluorochrome markers (See **Cell Staining Procedure** and **Expected Results** for further information on compensation). Contact Guava Technical Support for information regarding proven methods for inducing apoptosis in cell cultures.
4. For negative control samples, leave wells for untreated (non-induced) and unstained cells.
5. Culture cells in microplates to induce the required amount of apoptosis.

### For Suspension Cells:

6. *Optional.* Count cells using Guava ViaCount reagent (see following note)
7. Using a multichannel pipettor, add 100 µL of 1X PBS to each well. Centrifuge the microplate at 300 x g for 5 minutes at room temperature. Carefully remove the supernatant from each well. To minimize cell loss, do not remove all the supernatant.
8. Resuspend cell in 100 µL of culture medium\*. Mix cell suspensions by gently vortexing or titration. (\*As previously mentioned, this is a live cell assay measuring the dynamic process of apoptosis. We have found that cells resuspended in PBS for the 1 hour staining procedure demonstrated an artificially accelerated rate of apoptosis. This effect was apparent when results from replicate wells of a 96-well plate were compared. By comparison with staining reactions performed in culture media, those run in PBS showed a dramatic change in % apoptotic and % dead cells over sample acquisition time (40–50 minutes/plate).)
9. Proceed to **Cell Staining Procedure**.

**NOTE:** Cells can be counted using the Guava ViaCount assay. ViaCount also provides a good estimate of the number of apoptotic cells in your sample. From the ViaCount results, you can determine whether cultures are suitable for proceed-

ing with cell staining or have progressed beyond the desired stage of apoptosis. Alternatively, if the level of apoptosis is sub-optimal, you can make adjustments to the culture conditions or incubate the cells in the inducing agent longer. Refer to the Guava ViaCount Reagent package insert and *Guava EasyCyte User's Guide* for detailed information about this assay.

### For Adherent Cells:

6. Harvest apoptotic cells that may have detached into the culture medium by transferring the medium from each well to a new 96-well plate.
7. Rinse sample wells with 100 µL 1X PBS then transfer rinse to appropriate well from step 1 above.
8. To detach remaining cells, dilute Guava ViaCount CDR (Cell Dispersal Reagent) at 1:3 with PBS then add 100 µL of diluted CDR to each sample well and incubate for 3–5 minutes in a 37°C CO<sub>2</sub> incubator. (We recommend using Guava ViaCount CDR over other enzymatic treatments for detaching adherent cells and dispersing cell clumps since it contains multiple enzymes and is gentler than other treatments. Alternatively, cells may be dislodged mechanically or using EDTA.)
9. Add 100 µL 1X PBS to each well and pipet repeatedly to dislodge all cells.
10. Centrifuge both microplates at 300 x g for 5 minutes at room temperature. Carefully remove supernatant from each well. To prevent cell loss, do not remove all supernatant. Resuspend cells in each well in 50 µL culture media then pool appropriate wells for 100 µL total sample volume.
11. *Optional.* Count cells using Guava ViaCount reagent
12. Proceed to **Cell Staining Procedure**.

**Note:** Alternatively, adherent cells can be cultured in flasks, detached by appropriate means then transferred to 96-well plates for caspase assays.

## 7. CELL STAINING PROCEDURE

The following procedure for cell staining is a guideline. Different cell types may respond differently to various treatments for apoptotic induction. The level of activated Caspase 3/7 present in a cell can vary with apoptotic progression. You may need to adjust the amount of FLICA reagent and PI for optimal staining of your cell samples.

1. Add 10 µL of 10X FLICA reagent to each well. To mix, immediately pipet the cell samples up and down several times, changing tips between wells to avoid cross contamination. Remember to leave one negative control (untreated) well and one positive control (**PI single color control**) well unstained. These will be used for adjusting instrument settings.
2. Incubate for 1 hour at 37°C and 5% CO<sub>2</sub>. Protect samples from light exposure. Gently resuspend the cells twice during this incubation period by pipetting up and down to ensure an even distribution of FLICA reagent among all cells.
3. Carefully add 100 µL of 1X Wash Buffer to each well.
4. Centrifuge the microtiter plate at 300 x g for 5 minutes.
5. Carefully remove the supernatant from each well. To minimize cell loss, do not remove all the supernatant.
6. Repeat steps 4–5 one more time (two washes total).
7. Prepare a master mix with propidium iodide diluted 1:200 in 1X Wash Buffer. Prepare a sufficient quantity of master mix to label all reaction wells using 150 µL per well. To label an entire plate (96-wells), dilute 75 µL PI in 15 mL 1X Apoptosis Wash Buffer. Add 150 µL master mix to each well, changing tips be-

tween wells to avoid cross-contamination. Pipet several times to mix. Incubate samples for 5 minutes at room temperature. Remember to leave the negative control well and one of the FLICA-stained positive control wells unstained for PI (FLICA single color control). Also, make sure the PI single color control well is stained with PI.

8. Cell samples are ready for data acquisition on the Guava EasyCyte.

**NOTE:** Guava EasyCyte Caspase 3/7 is a live cell assay and samples should be acquired on the Guava EasyCyte within 90 minutes of completing the sample preparation. Stained samples from some cell lines may be fixed and stored for data acquisition the next day with little or no loss of fluorescent signal. However, not all cell types can be fixed and stored for extended periods of time. We recommend performing data acquisition within 1-2 hours after staining until proven fixation conditions have been established for your samples. Contact Guava Technical Support for additional information.

## 8. SAMPLE ACQUISITION

Samples are acquired on the Guava EasyCyte system using CytoSoft software. The Guava ExpressPlus application displays acquired data in 3 plots (dot-plots or histograms) allowing a choice of three fluorescence parameters in combination with forward scatter (FSC) to identify cells with specific phenotypic markers or properties. For the Caspase 3/7 kit, the use of the FSC channel for size and morphological discrimination, in combination with the PM3 and PM1 channels for fluorescent characteristics (FLICA and PI, respectively), is recommended.

Given that Caspase 3/7 staining results can vary greatly with cell type and mode of induction, we recommend adjusting the instrument settings prior to each sample set run. Such adjustments can best be performed using a non-induced/unstained sample (for FSC and PMT Voltage parameters) and single stained positive control samples (for Compensation of the FLICA/PI fluorochrome combination). The parameters and settings depicted here represent findings for one cell type (Jurkats) and should serve as a qualitative guideline for proper instrument adjustment. For further information regarding instrument setup or additional examples, please consult the Guava EasyCyte User's Guide.

1. Prepare a worklist to define the sample set that is to be analyzed.
2. Click on ExpressPlus, load your plate, open worklist, and start a new data-file.
3. Go to adjust settings. Click on the well containing the unstained cells.
4. While ExpressPlus software provides significant flexibility in data presentation, to perform initial instrument settings adjustments for the Caspase 3/7 assay, we recommend setting plot parameters as follows (See Figure 1).
  - Plot 1 - Dot Plot, FSC vs. PM3
  - Plot 2 - Dot Plot, PM1 vs. PM3
  - Plot 3 - Dot Plot, PM2 vs. PM3



Figure 1. Adjust Settings Window for Guava CytoSoft ExpressPlus Software.

5. Use the FSC Gain settings to reduce/amplify the FSC signal so that cells are visible and on-scale. Adjust FSC gain settings such that the predominant cell population of the untreated sample (live cells) is positioned between  $10e3$  and  $10e4$  on the FSC axis in Plot 1.
6. Exclude unwanted cellular debris by adjusting the FSC Threshold Marker (dotted red line in Plot 1 of Figure 1). When setting the threshold, consider debris to be any events occurring 1 log unit below the predominant cell population. However, leave enough debris to ensure that you are not excluding cell events. Any debris that is acquired can be excluded during analysis using a dot plot gate.
7. If samples contain high amounts of debris, you may wish to exclude these events from the total event count through use of a Gate in Plot 1. Debris can be excluded during acquisition by checking the **Enable Regions** box above Plot 1. Events above the FSC threshold falling within the rectangular gated region are displayed and analyzed on Plots 2 (and 3) provided that **P1** is checked in the **Gated On** box above each plot. If **Enable Regions** is not selected, all of the events collected above the FSC threshold will be displayed and analyzed on Plots 2 and 3.
 

**Note:** You can only select a count gate from the **Adjust Settings** screen. If no gate is specified, the count gate will default to all events collected above the FSC threshold in Plot 1.
8. Adjust the **PMT voltages** so that the unstained population is positioned in the lower-left quadrant (between  $10e0$  and  $10e1$ , see Figure 2) for all fluorescent channels. Adjust the PMT settings by starting from a lower voltage setting and gradually increasing the voltage.

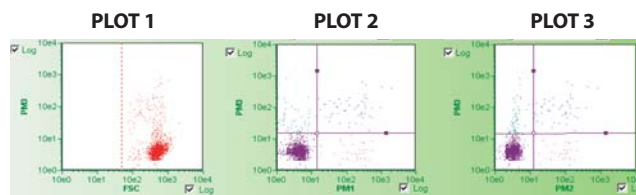


Figure 2. Proper PMT voltage settings position the unstained cell fraction (viable Jurkat cells) in the lower left quadrant for all three PM channels

9. After adjusting PMT voltage settings, click **Next Step** and proceed to compensation adjustment.
10. In order to optimize the data analysis, it is recommended that an initial compensation be performed on the fluorochromes applied within each sample set. Click on Adjust Settings again. Select sample with FLICA stain only. Adjust the settings (**PM1-%PM3**) to correctly align the FLICA-positive and negative fractions along the y-axis. Once finished, click Next Step.

11. Click on Adjust settings again. This time choose the PI-positive single stain control. Perform compensation for this channel by adjusting the **PM3-%PM1** to correctly align the PI-positive and negative fractions along the x-axis. Click Next step. Select adjust settings again. Now chose a dual stained positive control and verify the adjustments by assessing separation of the 4 populations as shown. Depicted below are examples of the PM1 (PI) vs PM3 (FLICA) dot plot showing separation of the 4 populations present in a Jurkat sample following apoptosis induction. Ideally, adjust the settings until the centers of the positive and negative populations (for each individual stain) are roughly equal. However, due to the fact that dead cells will be dimly stained with FLICA and not completely negative, the dead fraction (PI (+)/FLICA (dim)) is properly aligned when positioned just off parallel (**Plot A**).

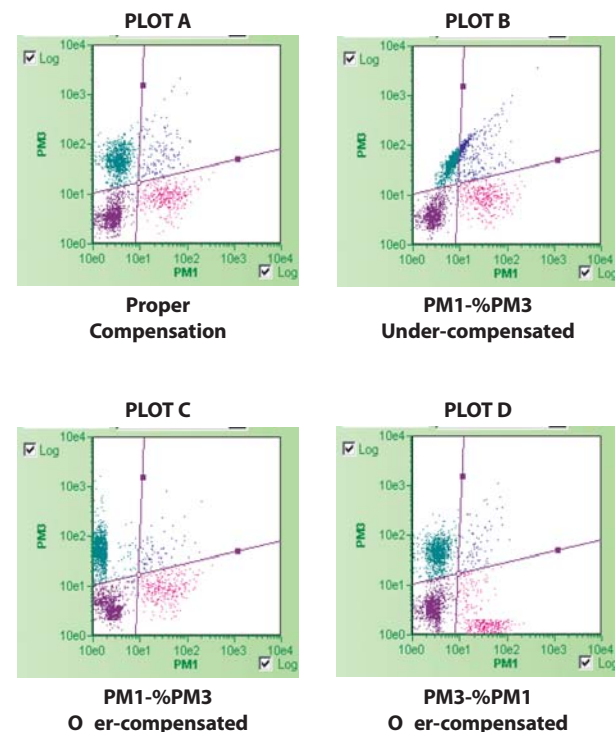


Figure 3. Adjusting instrument settings for proper 2-color compensation. Depicted in Plots A-D are results from collecting an induced Jurkat sample (stained with the Caspase-3/7 reagents) at several different compensation values.

12. Click Resume to acquire all samples at desired settings.

## 9. EXPECTED RESULTS

Using the ExpressPlus application, data derived from samples stained with Guava EasyCyte Caspase 3/7 Kit reagents can be displayed in 3 plots — **Plot 1**, FSC vs FLICA (PM3); **Plot 2**, PI (PM1) vs FLICA (PM3); and **Plot 3**, a histogram depicting the Caspase 3/7 (FLICA) positive events (Figure 4). Events to be analyzed can be gated to remove undesirable debris events by checking the **Enable Regions** box above Plot 1 and then properly positioning the rectangular gating box around the desired events. Events above the FSC threshold falling within the rectangular gated region are displayed and analyzed in Plots 2 and 3. If **Enable Regions** is turned off (box unchecked), all events above the FSC threshold will be displayed and analyzed.

Analyze all data generated using quadrant markers such that all 4 populations are separated. The labeled figure below clearly shows what each of the 4 populations are. If desired, statistical data can be obtained from both the dot plot and the histogram. It is important to note that when setting histogram markers, allowing for **Overlapping Markers** will result in the events falling within the overlapping region being counted twice in the statistics.

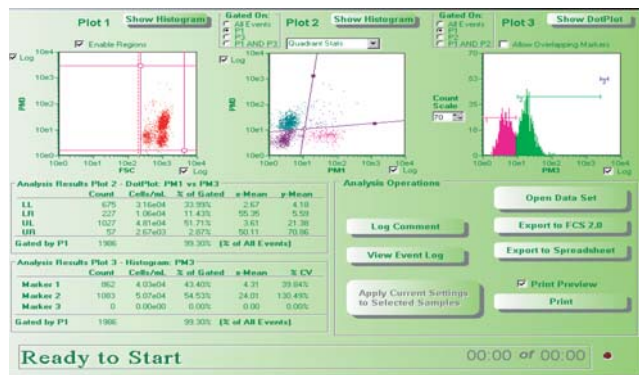


Figure 4. Representative ExpressPlus analysis screen shot for Caspase 3/7 assay.

The Guava ExpressPlus software calculates and presents results automatically in the Plot 2 and Plot 3 Analysis Results table. Statistical data (histogram and dot plot) include the cell count, cell/mL, mean signal intensity (x- and y-Mean), and % CV (for histogram data) for all the data in the plot (if ungated) or the subset of data within the markers/gate. Additionally, the data is expressed as a percentage of the total data within the plot (whether gated from the dot plot or histogram), and as a percentage of data within 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 either FCS 2.0- and 3.0-compatible third party software.

The assay results can vary with cell type, induction method, and conditions used. Figures 5-7 illustrate examples of results we obtained using the Guava EasyCyte Caspase 3/7 Kit with cell samples under various conditions.

Figure 5 shows the results of staining an untreated Jurkat sample with the Caspase 3/7 Kit. As expected, the vast majority of cells appear live.

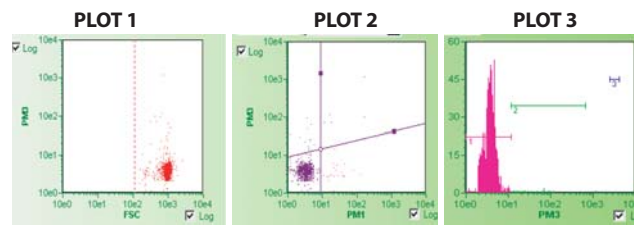


Figure 5. Untreated Jurkat cell samples demonstrate a low background level of apoptosis in the Guava Caspase 3/7 Assay.

The Quadrant markers in Fig. 5, **Plot 2** distinguish the non-apoptotic live cells in purple (PI (-)/FLICA (-)) from the mid-apoptotic population in teal green (PI (-)/FLICA (+)) and late apoptotic/dying fraction in blue (PI (+)/FLICA (+)). Dead cell events in pink (PI (+)/FLICA (dim/-)) appear in the lower right quadrant of the dot plot. In Fig. 5, **Plot 3**, the frequency histogram of the number of events (y-axis) vs. FLICA stain intensity (x-axis) shows two peaks: caspase 3/7 positive cells (Marker 2) and caspase-3/7 negative (Marker 1) cells.

Figure 6 shows Jurkat cells rapidly undergoing apoptosis when treated with staurosporine.

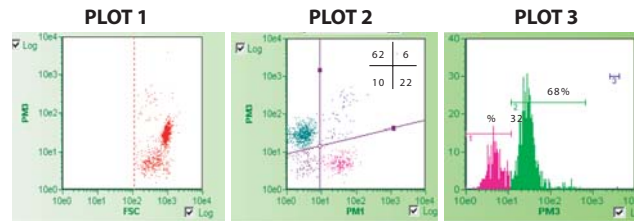


Figure 6. Jurkat cells treated with 1 μM staurosporine for 3 hours, as analyzed using the Guava Caspase 3/7 assay.

Within 3 hours, only a small fraction of the cell sample (purple cells, 10%) remains uncommitted to the apoptotic pathway while nearly 70% stained positively for Caspase 3/7 activity. From Plot 2, the pink fraction (PI (+)/FLICA (-)), representing 22% of the sample, is composed of cells that were dead prior to induction or that have died via the apoptotic pathway. Very few late apoptotic/dying cells appear in this population. By contrast, under similar induction conditions, HeLa cells demonstrate different kinetics of apoptotic progression than the Jurkat cell line. Figure 7 shows about 50% of the sample HeLa population to be in mid-phase apoptosis (shown in teal green), 23% late apoptotic (blue), and 2% dead (pink events).

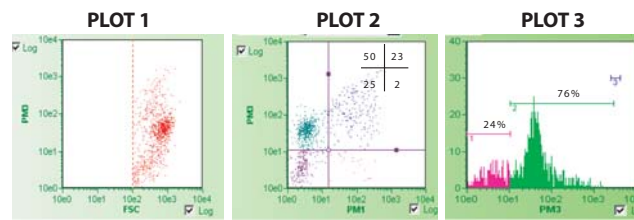


Figure 7. Serum starved HeLa cells induced for apoptosis (1 μM staurosporine for 4 hours)

## 10. TROUBLESHOOTING

1. Avoid excessive exposure of cell samples to light to prevent fading of fluorochromes.
2. Dim or false negative staining with the FLICA reagent can also indicate reagent degradation. Verify proper storage and handling of the FLICA reagent and 250X stock solutions. Always prepare a fresh FLICA working solution just prior to cell staining.
3. Acquire data from samples as soon as possible after staining. Guava EasyCyte Caspase 3/7 is a live cell assay; therefore the staining profiles can change as the cell sample ages. Guava recommends acquiring data within 1–2 hours after completing staining.
4. If the final concentration of the stained sample is too high ( $> 5 \times 10^5$  cells/mL), the Guava EasyCyte may not yield accurate results. Dilute sample further with 1X Wash Buffer (or PBS) to bring cell concentration to the recommended range ( $1 \times 10^4$ – $5 \times 10^5$  cells/mL).
5. The default number of events to acquire is 5000. We recommend acquiring 2000 events/sample, after gating out debris, to ensure an adequate number of cellular events. You can acquire more or less events but your statistical error increases as acquisition number decreases. For this reason, it is important to ensure that debris is either properly gated out during acquisition or sufficient events are captured to accommodate debris' contribution to the total cell count.
6. Check the mixing option has been selected in the worklist to ensure accurate readings.
7. Run Guava Check™ (catalog No. 4500-0020) to verify proper instrument function and accuracy.
8. To prevent a buildup from cell debris in the flow system, run Quick Clean using deionized water following acquisition of 12–24 samples. If your samples contain significant debris, run Quick Clean with Guava ICF followed by water.
9. If you are acquiring data from a sample but the Cell Count number is not increasing and the Events to Acquire bar is not moving, there is probably either insufficient volume to continue acquiring sample or a blockage of the flow system. First, check sample volume by pausing the CytoSoft application and ejecting the tray. If the sample volume is below 50 μL (for either 96-well or microfuge tube formats), there is insufficient sample volume for the instrument to acquire. Either add additional volume or proceed to the next sample. If sample volume is greater than 50 μL, then the lack of events is probably due to a clog. Cell aggregates, cell debris, bleach crystals, or other particulates can cause a clog or blockage of the flow system. Click **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.
10. If events appear off-scale in either the Dot or Histogram Plots it is possible that the FSC gain, PM1, PM2, or PM3 voltages were set incorrectly during initial settings adjustment. Alternatively, the samples may be stained too brightly. To correct, check and readjust settings on the positive control sample in **Adjust Settings** to be sure that they allow for bright signals (see Figure 1).
11. If you find there is poor resolution between the negative and positive populations for a given stain (FLICA and/or PI), a number of potential explanations, and methods of correction, may exist.

- Fluorescent probes were stored improperly or exposed to light. (See **Handling and Storage** section).
- The voltages were set to low to detect fluorescent signals. Adjust the settings to increase the fluorescent signal.
- Staining is either incomplete or non-specific binding has occurred. You may need to optimize the reagent concentration for your cell type by performing a titration. For Jurkat cells, we have found that the reaction and reagent volumes listed in the Cell Staining Procedure showed little variation in % apoptotic cells across a wide range of cell concentrations ( $5 \times 10^4$ - $5 \times 10^5$  cells/mL). You may also wish to employ an additional wash step to remove non-specifically bound label.
- There is a high degree of background noise. Increase the FSC threshold or use the Enable Regions parameter in Plot 1 to gate out debris.

12. Poor resolution may also occur between positive populations in plots displaying PM1 and/or PM3. In addition to the causes and solutions listed in #11, such instances may result from improper initial settings adjustments. Pause the sample acquisition and re-adjust settings first using the single stain controls and then a dual stained sample. If the voltage was too high, causing signal to bleed into the other parameter, reduce the voltage or adjust compensations settings to separate the populations.

13. For more troubleshooting tips, refer to the *Guava EasyCyte System User's Guide* or contact Guava Technical Support.

## 11. LIMITATIONS

1. The Guava EasyCyte yields optimal results when the stained cell sample for data acquisition is between  $1 \times 10^4$  to  $5 \times 10^5$  cells/mL. To obtain the most accurate assay results, adjust the concentration of the cell samples to within the recommended range.
2. Guava Caspase 3/7 assay reagents are formulated to meet most assay requirements. Modification of the staining protocol and reagent concentration(s) may be necessary to ensure optimal performance for individual assays.

## 12. REFERENCES

1. Salvesen GC, Dixit VM. Caspases: intracellular signaling by proteolysis. *Cell*, 1997; 91:443-446.
2. Slee EA, et al. Serial killers: ordering caspase activation events in apoptosis. *Cell Death and Differ.*, 1999; 6:1067-74.
3. Alnemri ES, et al. Human ICE/CED-3 protease nomenclature. *Cell*, 1996; 87:171.
4. Ekert PG, et al. Caspase inhibitors. *Cell Death and Differ.*, 1999; 6:1081-1086.

## 13. 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.

## 14. RETURNED GOODS POLICY

Please inspect package(s) upon receipt and inform us immediately of any shortage

Guava® EasyCyte Caspase 3/7 Kit

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