Guava Technologies

4600-0790
rev B

Guava® Cell Cycle Reagent

Cat. No.4500-0220
100 Tests

To determine the percentage of cells in G0/G1, S and G2/M phases based on DNA content

Research Use Only
Not for Use in Diagnostic Procedures

1. PRODUCT DESCRIPTION AND INTENDED USE

The cell cycle describes the process of the replication and division of chromosomes within the nucleus, which occurs prior to cell division. Cancer cells develop when the normal mechanisms for regulating cell cycle are disrupted. It is important to identify the genetic basis for this disruption and to develop therapies to preferentially target those cells with abnormalities. One of the ways to screen for potentially therapeutic drugs, or the effects of specific genes on cell cycle, is to measure changes in cell cycle kinetics under varying conditions. For cells to divide they must first duplicate their nuclear DNA. By labeling cellular DNA with propidium iodide (PI) you can discriminate cells in different stages of the cell cycle. Resting cells (G0/G1phase) contain two copies of each chromosome. As cells progress toward mitosis, they synthesize DNA (S phase), allowing more PI intercalation with a resulting increase in fluorescence intensity. When all chromosomes have replicated and the DNA content has doubled (G2/M phase), the cells fluoresce with twice the intensity of the G0/G1 population. The G2/M cells eventually divide into two cells. Cells can be fixed, permeabilized and stained with PI according to the protocol below. Data from the stained cells are acquired on the Guava® PCA™, Guava PCA-96, Guava PCA-96 AFP, Guava EasyCyte™, or Guava EasyCyte Mini using CytoSoft™ software. Data are displayed in a single parameter histogram. Four markers are available to analyze the various populations including the optional fourth marker to discern apoptotic cells, cell aggregates, or an internal standard. Statistics for each population within the histogram in CytoSoft include percentage of total, and PM2 mean, median, and %CV of fluorescence intensity.

The Guava Cell Cycle data for all samples within a data set are saved to an FCS 3.0 file, and optionally to individual FCS 2.0 files. The data can be analyzed immediately after the sample is acquired or recalled later. In addition to the saved FCS data file, all results and the acquisition information are exported to a comma separated values (CSV) spreadsheet file.

CytoSoft does not apply any sophisticated “curve-fitting” algorithms to the data. In addition to their other benefits, curve-fitting algorithms can compensate for the presence of aggregates which include doublets of G0/G1 cells that fluoresce as brightly as G2/M cells. If desired, a third-party curve-fitting software package such as ModFit™ or MultiCycle can apply more sophisticated analysis algorithms to the data, and thus provide a more accurate assessment of the percentage of cells in each phase and their relevant statistics. However, for most applications, CytoSoft is sufficient for assessment of the number of cells in each phase.

2. MATERIALS PROVIDED

- Guava Cell Cycle Reagent (Catalog No. 4500-0220, 100 tests)

3. HANDLING AND STORAGE

1. The Guava Cell Cycle Reagent should be stored refrigerated (2 to 8°C). Do not freeze. Refer to the expiration date on the package label.
2. Do not use the reagent after the expiration date.
3. The Guava Cell Cycle Reagent contains light-sensitive dyes. Shield from excessive exposure to light.

4. WARNINGS AND PRECAUTIONS

1. The Guava Cell Cycle Reagent is intended for research use only and not for use in diagnostic procedures.
2. Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using this reagent.
3. The Guava Cell Cycle Reagent contains dye that may be carcinogenic and/or mutagenic. Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic and mutagenic reagents.
4. The Guava Cell Cycle Reagent contains sodium azide, which is toxic. Contact with acids liberates toxic gas. Flush plumbing with copious amounts of water when disposing of azide compounds to avoid potentially explosive conditions arising from azide deposits in pipes.
5. Avoid microbial contamination of the solution, which may cause erroneous results.
6. All biological specimens and materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
7. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
8. Material Safety Data Sheets (MSDS) for kit reagents are available from our web site (www.guavatechnologies.com) or by contacting Guava Technical Support (support@guavatechnologies.com; telephone 866-448-2827 or 510-576-1400).

5. EQUIPMENT AND MATERIALS REQUIRED

- Guava instrument with Cytosoft software 2.0 or above
- Guava Cell Cycle Reagent (Cat. No. 4500-0220)
- Cell suspension(s)
- Ethanol 70%
- 1X PBS
- Medium appropriate for your cells
- For 96-well plate assays: V-shaped, 25-mL and/or 55-mL troughs (25 mL, Apogent Discoveries Cat. No. 8093 and 55 mL VWR Cat. No. 210070-970).
- For 96-well plate assays: 96-well microplate plates, round bottom (Falcon #353910, #353918), flat bottom (Falcon #353075, #353915), or hydrogel-coated flat bottom (Costar #3474, Myriad Industries #0400-3474) or equivalent. Refer to the Guava instrument's user's guides for compatible microplates.
- Micropipettors, single, 8 or 12 channel (the latter two for 96-well plate assays)
- Disposable micropipettor tips, sterile preferred
- 15- or 50-mL conical tubes, sterile preferred
- 12 x 75 mm polystyrene tubes (VWR Cat. No. 60818-270)
- Microcentrifuge tubes without screw caps, 1.5 mL (VWR Cat. No. 20170-201) or with screw caps, 1.5 mL (VWR, Cat. No. 20170-704 or equivalent)
- For Guava PCA-96, Guava PCA-96 AFP and Guava EasyCyte: 0.5-mL microcentrifuge tubes (VWR Cat. No. 20170-210) for sample acquisition
- Vortex mixer
- Centrifuge, with 96-well microplate holders, if preparing cells in microplates
- Disposable gloves
- Guava ICF™ Instrument Cleaning Fluid (Catalog No. 4200-0140)
- 20% bleach solution
- Deionized water
- For Guava PCA and Guava EasyCyte Mini: Guava ViaCount® Reagent (Cat No. 4000-0040) or ViaCount Flex (Cat No. 4500-0110) (optional)
- For Guava PCA-96, Guava PCA-96 AFP and Guava EasyCyte: Guava PCA-96 ViaCount Flex Reagent (4700-0060) (optional)
- Guava CDR™ (Cat No. 4700-0050) (for adherent cells, optional)

6. REAGENT AND SAMPLE PREPARATION

Assay Considerations

Staining cycling cells using the recommended protocol and data acquisition on the Guava instruments can usually be completed within one to two hours, depending on the number of samples and the cell concentration. However, prior to the staining, cell fixation requires at least one to twelve additional hours. Additional time (hours to days of culture or pre-treatment with test compounds) may be required as well to prepare the cells, depending on the particular test conditions.

It is important to remove the ethanol used for fixation before adding the Guava Cell Cycle Reagent. However, in some cases, depending on the cell line, the PBS washing step to completely remove the ethanol can be eliminated. Removal of ethanol and/or washing in PBS should be done in either 12 x 75 mm tubes or 96-well round bottom plates but not in 1.5-mL microcentrifuge tubes because of substantial cell lose due to cells sticking to the tube. If cells are cultured in 96-well flat bottom plates, they should be transferred into 96-well round bottom plates for ethanol fixation, washing and staining.

A typical assay uses 200 µL of Guava Cell Cycle Reagent to stain 1 x 10^5 to 2 x 10^5 fixed cells per assay condition. When acquiring samples on the Guava instrument, if the cell count is higher than 1200 particles per µL, the software will display a high particle concentration warning. The sample should then be diluted with the Guava Cell Cycle Reagent until the cell concentration is under 1200 particles per µL.

Cell Sample Preparation

Prepare a negative control sample. The negative control should be a sample from your cell culture in log phase growth and not treated with any drug. If desired, prepare a positive control sample that has been treated with a compound known to arrest your cells in a particular phase of the cell cycle. Prepare both the negative and positive control samples as described below for the test samples.

Culturing Cell Samples

A. Culturing non-adherent samples grown in 96-well microplates

NOTE: You can follow this protocol to culture and harvest adherent cells in 96-well plates; however you must use plates coated with a material such as hydrogel that will prevent the attachment of the cells to the plastic. Guava also

recommends that you validate that your adherent cells grow similarly unattached in hydrogel coated plates as they go attached in regular (uncoated) plates. If that is not the case, please see sections B and C below for how to assess cell cycle with adherent cells.

1. Prior to adding cells to the microplate, determine the cell concentration of the stock using Guava ViaCount Reagent (for cells at concentrations >2 x 10^5 cells/mL) or Guava ViaCount Flex Reagent (for cells at concentrations >1 x 10^4 cells/mL). Please see the package inserts for instructions in how to use those products to determine cell concentrations.
2. Add 1 x 10^5 to 2 x 10^5 cells in a 200 µL volume (between 5 x 10^5 cells/mL and 1 x 10^6 cells/mL) to each well of a microplate with or without treatments.
   - If adding agents which arrest the cell cycle, cells can first be synchronized in G0 by culturing cells for 24 to 36 hours in basal medium without serum. Synchronization can be performed with a stock of cells grown in a T-flask prior to harvesting and adding the cells to the 96-well plate.
   - After synchronization, make stocks of test agents in medium with serum and add 20 µL of those stock reagents to the appropriate wells of the microplate.
3. Culture further, if necessary, per your own protocols.
4. Proceed to *Cell Fixation in a 96-well plate*.

B. Culturing and harvesting adherent cells in 24-well tissue culture plates

1. Prior to adding cells to the plate, determine the cell concentration of the stock using Guava ViaCount Reagent (for cells at concentrations >2 x 10^5 cells/mL) or Guava ViaCount Flex Reagent (for cells at concentrations >1 x 10^4 cells/mL). Please see the package inserts for instructions on how to use those products to determine cell concentrations.
2. Add 50,000 adherent cells in 2 mL of medium to each well of a 24-well tissue culture plate.
3. Incubate the cells at 37°C to allow the cells to attach to the plate and to grow to 60-80% confluency before proceeding.
   - If adding agents which arrest the cell cycle, cells can first be synchronized in G0 by culturing cells for 24 to 36 hours in basal medium without serum.
   - After synchronization, make stocks of test agents in medium with serum and add 20 µL of those stock reagents to the appropriate wells of the microplate.
4. Culture further, if necessary, per your own protocols.
5. Harvest the cells from the 24-well plates following standard protocols. Adherent cells can be removed using Guava CDR instead of other enzymatic treatments as follows:
   - Label 15-mL tubes for each well containing test and control cells.
   - Remove the culture medium from each well and add to each appropriate 15-mL conical tube.
   - Rinse the wells once with 1 mL/well of 1X PBS. Add the PBS into the same appropriate 15-mL conical tube.
   - Dilute the Guava CDR 1:3 with PBS.
   - Add 0.5 mL of diluted Guava CDR to each 24 well plate and incubate for 3-5 minutes in a 37°C incubator, or until cells just begin to detach.
   - Add 2 mL of media with serum and pipet repeatedly to release cells from the well bottom.
   - Pipet the Guava CDR and media into the appropriate 15-mL conical tube as above.
   - Add 1mL of 1X PBS to rinse any remaining cells. Transfer the 1X PBS from each well to the appropriate 15-mL conical tube.
6. Centrifuge the tubes at 300 x g for 5 minutes.
7. Aspirate and discard the supernatant.
8. Add 500 µL of medium or PBS to each 15-mL conical tube. Mix the harvested cell sample by vortexing or pipetting repeatedly to ensure a homogenous suspension.
9. Determine the concentration of the cell samples. If necessary, adjust the cell samples to between 5 x 10^5 and 1 x 10^6 cells/mL using medium or PBS.
10. Add 200 µL per well of the cell sample to each well of a 96-well round bottom plate. Samples from one 24 well are typically sufficient for 3 replicates in a 96-well plate.
11. Proceed to *Cell Fixation in a 96-well plate*.

C. Culturing and harvesting samples which have been grown in tissue culture vessels other than 96- and 24-well plates

This procedure can be used for both non-adherent and adherent cells.

1. Prior to adding cells to the flasks, determine the cell concentration of the stock using Guava ViaCount Reagent (for cells at concentrations >2 x 10^5 cells/mL) or Guava ViaCount Flex Reagent (for cells at concentrations >1 x 10^4 cells/mL). Please see the package inserts for instructions in how to use those products to determine cell concentrations.
2. Add the cells to the flasks at low concentrations to ensure log phase growth.
3. Incubate the cells at 37°C. If using suspension cells, use within 2 days of subculturing. If using adherent cells, allow the cells to attach to the plate and to grow to 60-80% confluency before proceeding.
   - If adding agents which arrest the cell cycle, cells can first be synchronized in G0 by culturing cells for 24 to 36 hours in basal medium without serum.
   - After synchronization, make stocks of test agents in medium with serum and add those stock reagents to the flasks.
4. Culture further, if necessary, per your own protocols.
5. Harvest the cells from the culture vessels following standard protocols. If using adherent cells, they can be removed using Guava CDR instead of other enzymatic treatments as follows (volumes indicated below are for harvesting cells from a T75 flask and should be scaled appropriately for other sizes of culture vessels):
   - Remove the culture medium and add to a 50-mL conical tube.
   - Rinse the flask once with 5 to 10 mL of PBS. Add the PBS into the same conical tube above.
   - Dilute the Guava CDR 1:3 with PBS.
   - Add 3 mL of diluted Guava CDR and incubate for 3-5 minutes in a 37°C incubator, or until cells just begin to detach.
   - Add 6 mL of media with serum and pipet repeatedly to release cells from the flask bottom.
   - Pipet the Guava CDR and media into the same conical tube.
6. Mix the harvested cell sample by vortexing or pipetting repeatedly to ensure a homogenous suspension.
7. Determine the concentration of the cell sample. If necessary, adjust the cell sample to between 5 x 10^5 and 1 x 10^6 cells/mL using medium or PBS.
8. If conducting the assay in 96-well format, add 200 µL of each cell sample with a multi-channel micropipettor to each well of a round bottom plate. Proceed to *Cell fixation in a 96-well plate*.
9. If conducting the assay in tubes, proceed to *Cell fixation in a 50-mL tube*.

Cell Fixation

NOTE: It is important to have a single cell suspension prior to fixation. Otherwise the fixation process will result in a high percentage of doublet cells that will decrease the accuracy of the results.

A. Cell fixation in a 96-well plate

1. Transfer the cell sample from the 96-well flat bottom or 24-well plate to a 96-well round bottom plate if the cells are not already in a round bottom plate.
2. Centrifuge the cells at 450 x g for 5 minutes with the brake on low and at room temperature.
3. Remove and discard the supernatant being careful not to touch the pellet.
4. Add 200 µL of 1X PBS to each well using a multi-channel pipettor.
5. Mix the cells in the well by pipetting up-and-down several times.
6. Centrifuge the cells in the round bottom plate at 450 x g for 5 minutes with the brake on low and at room temperature.
7. Remove and discard the supernatant.
8. Using a multi-channel pipettor, thoroughly resuspend the cells by repeat pipetting in the residual PBS, pipetting up and down several times.
9. Place the round bottom plate containing the resuspended cells on a lab shaker.
10. Add 200 µL of ice-cold 70% ethanol dropwise into the wells while shaking at low speed (speed 3).
11. Seal the plate with a microplate sealer and refrigerate cells for at least one and up to 12 hours prior to staining. Fixed cells are stable for several weeks at 4°C and for two to three months at –20°C
12. Proceed to *Cell Staining Protocol in 96-well format*.

B. Cell fixation in a 50-mL tube

1. Centrifuge the tube at 450 x g for 5 minutes with the brake on low.
2. Remove and discard the supernatant.
3. Add 25 mL of 1X PBS to each tube.
4. Mix the cell sample by vortexing or pipetting repeatedly to ensure a homogenous suspension.
5. Centrifuge the tube at 450 x g for 5 minutes with the brake on low.
6. Remove and discard the supernatant leaving approximately 500 µL of 1X PBS.
7. Resuspend the cells thoroughly in the residual PBS by vortexing or repeated pipetting.
8. Add the resuspended cells dropwise into a 50-mL conical tube containing 30 mL of ice-cold 70% ethanol while vortexing on medium speed (setting at 5).
9. Refrigerate the cell preparation for at least one and up to 12 hours prior to staining. Fixed cells are stable for several weeks at 4°C and for two to three months at –20oC.
10. Proceed to *Cell Staining Protocol in 96-well format or tubes*.

Cell Staining Protocol

A. Cell staining in 96-well format

1. Warm Guava Cell Cycle Reagent to room temperature; shield from excessive light exposure. Warm 1X PBS to room temperature.
2. Transfer 200 µL of the samples into the wells of a 96-well round bottom plate if the samples have not yet been transferred.
3. Centrifuge the 96-well round bottom plate containing the samples at 450 x g for 5 minutes with the brake on low and at room temperature.
4. Remove and discard the supernatant being careful not to touch the pellet. After centrifugation, the well should contain a visible pellet or a white film on the bottom of the plate.

5. Using a multi-channel pipettor, add 200 µL of 1X PBS to each well. Mix cells in the wells by pipetting up and down several times. Let the plate stand at room temperature for one minute.
6. Centrifuge the 96-well round bottom plate at 450 x g for 5 minutes with the brake on low and at room temperature.
7. Remove and discard the supernatant being careful not to touch the pellet.

   NOTE: The PBS washing step (steps 5 to 7) can be omitted if the user has first shown that this wash step is not necessary to minimize the %CV of the G0/G1 peak. However, the cells should still be centrifuged once to remove the ethanol. The Guava Cell Cycle Reagent should not be added to cells still in ethanol.
8. Add 200 µL of Guava Cell Cycle Reagent to each well.
9. Mix by pipetting up and down several times.
10. Incubate the 96-well round bottom plate at room temperature shielding away from light for 30 minutes.
11. Acquire the samples on the Guava PCA-96, Guava PCA-96 AFP or Guava EasyCyte system.

B. Cell staining in tubes

1. Add 1 x 10^5 to 2 x 10^5 cells in 200 µL to 1 mL volume of ethanol-fixed cells to a 12 x 75 mm polystyrene test tube.

   NOTE: You must use a 12 x 75 mm tube instead of a 1.5 mL microcentrifuge tube due to a large amount of cell loss with the fixed Jurkat cells (and perhaps other cell lines) over time when stored in 1.5 mL microcentrifuge tubes.
2. Centrifuge ethanol-fixed cells at 450 x g for 5 minutes with the brake on low.
3. Remove and discard the supernatant.
4. Resuspend the cells in 1 mL 1X PBS.
5. Vortex cells and incubate for 1 minute.
6. Centrifuge at 450 x g for 5 minutes with the brake on low at room temperature.
7. Remove and discard the supernatant.

   NOTE: The PBS washing step (steps 4 to 7) can be omitted if the user has first shown that this wash step is not necessary to minimize the %CV of the G0/G1 peak. However, the cells should still be centrifuged once to remove the ethanol. The Guava Cell Cycle Reagent should not be added to cells still in ethanol.
8. Resuspend the cells in 200 µL Guava Cell Cycle Reagent.
9. Incubate at room temperature for 30 minutes, shielded from light.
10. Transfer all samples to 1.5-mL microcentrifuge tubes (Guava PCA or Guava EasyCyte Mini) or 0.5-mL microcentrifuge tubes (Guava PCA-96, Guava PCA-96 AFP or Guava EasyCyte system).
11. Acquire the data on the Guava instrument.

7. EXPECTED RESULTS

Figures 1a and 1b show typical results obtained with the Guava Cell Cycle Reagent. Log-phase Jurkat T cells were serum starved for 24 hours and treated with (Figure 1b) and without (Figure 1a) 0.029 µg/mL of nocodazole for an additional 24 hours in medium, ethanol fixed overnight and stained in 96-well plates according to the protocol described above.

The upper plot shows the distribution of the cell cycle phases (G0/G1, S and G2/M) in histogram format. The DNA Histogram Results show the result for the percentage of cells in G0/G1 (M1), S (M2) and G2/M (M3) under % Total. The bottom plot shows the Forward Scatter (FSC) versus DNA content of the cell sample.

Untreated Jurkat cells had 48.9%, 19.4% and 32.1% of cells in G0/G1 (M1), S (M2) and G2/M (M3), respectively. Jurkat cells treated with nocodazole for 24 hours had fewer cells in G0/G1 and S phases and many more in the G2/M phase (11.0%, 11.0% and 76.9% in G0/G1, S and G2/M, respectively). As expected, nocodazole, an anticancer drug that interferes with the structure and function of microtubules in interphase and mitotic cells, arrested cells in the G2/M phase. Thus, the Guava Cell Cycle Reagent and Assay can be used to detect differences between cycling and arrested cells.

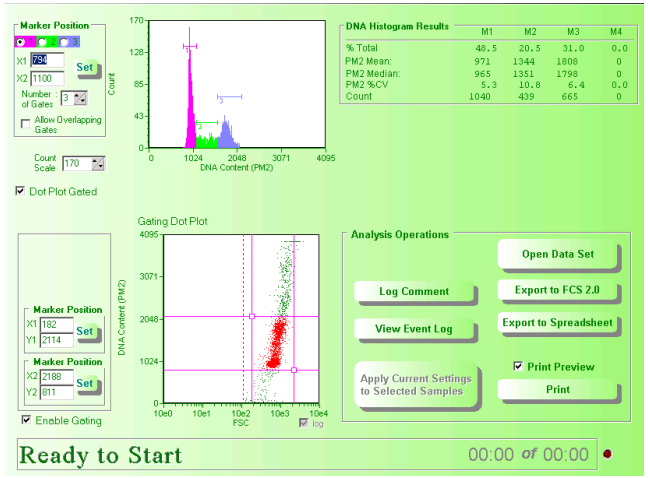

Figure 1a Untreated Jurkat cells

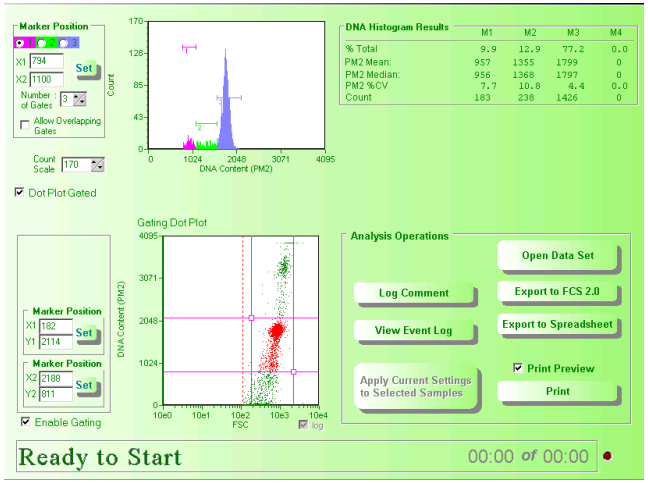

Figure 1b Nocodazole-treated Jurkat cells

Figures 1a and 1b. Examples of cycling and arrested cells analyzed using the Guava Cell Cycle Reagent and Assay. Untreated (Figure 1a) and nocodazole-treated (Figure 1b) Jurkat cells were prepared according to the above protocol and acquired on a Guava PCA-96 system. The histograms show the cells in G0/G1 (pink peak on left), S (green center peak) and G2/M (blue peak on right). In the dot plot, the cycling cells are shown within the rectangular gate and are in red. The cells excluded from the gate are shown in green and are either debris or sub-G0 cells (below the gate) or aggregates or G4 cells (above the gate).

For more quantitative results and sophisticated analysis of the percentages of cells in G0/G1, S, and G2/M phases, consult the Guava protocol note, "Using Modfit to Analyze the Results from the Guava® PCA™ System." The document will guide the user from exporting the data to an FCS 2.0 file to analyzing the results on ModFit, a third party curve-fitting software package.

8. TROUBLESHOOTING TIPS

1. Setting the FSC threshold too low may affect your results because cell debris will be included. Additionally, noise may appear in the PM2 histogram as a result. Try adjusting the threshold upward in order to reduce noise and debris or enable gating in analysis to exclude unwanted debris or other events.
2. Avoid excessive exposure of the stained samples to light.
3. If the concentration of the stained cell sample for data acquisition is low (<7 x 10^4 cells/mL), the Guava instruments will not be able to acquire 5,000 events in the allotted time for sample collection (10 min). Centrifuge the sample at 400 x g for 7-10 minutes and remove a sufficient amount of the supernatant to increase the cell concentration to >7 x 10^4 cells/mL.
4. The default number of events to acquire is 5000. You may input a different number, however, your statistical error may increase as you decrease the number of events for acquisition. You should not collect below 2000 gated events. Collecting below 2000 gated events may yield erroneous results.
5. Run Guava Check (Catalog No. 4500-0020) to verify proper instrument function and accuracy.
6. Be sure that samples are properly resuspended prior to acquisition. For the Guava PCA and Guava EasyCyte Mini, vortex samples just prior to acquiring. For the Guava PCA-96, Guava PCA-96 AFP and Guava EasyCyte systems, check that the mixing option has been selected in the Worklist file used to collect the data in the Guava Cell Cycle software application. Cells in the sample will settle quickly and your Guava Cell Cycle results (percent of cells in G0/G1, S and G2/M, etc.) will be inaccurate unless each sample is mixed prior to acquisition.
7. A Quick Clean will be performed at the end of every Worklist when using the Guava PCA-96, Guava PCA-96 AFP or Guava EasyCyte system. 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 after every 12 to 24 sample acquisitions. Alternately, if your samples contain significant amounts of cellular debris, run Quick Clean with Guava ICF followed by water, to prevent clogs or blockage. If you are acquiring samples on the Guava PCA or Guava EasyCyte Mini, you should perform manual Quick Cleans as described above.
8. 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 to acquire sample, or a blockage of the flow system. Check first for the lack of sufficient sample volume (on the Guava PCA-96, Guava PCA-96 and Guava EasyCyte, you must first pause the CytoSoft application and eject the tray). If the sample volume is below 50 µL, there is not enough sample for the instrument to acquire. Either add additional Guava Cell Cycle Reagent to bring the sample volume up to greater than 50 µL, or proceed to the next sample. If the sample volume is more than 50 µL, 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 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, refer to the appropriate Guava instrument user's guide, or contact Technical Service.

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

9. LIMITATIONS

1. The results of the assay are dependent upon proper handling of samples and reagents.
2. For accuracy, at least 2000 gated events should be collected.
3. If the particle per µL (p/µL) is greater than 1200, dilute the sample with Guava Cell Cycle Reagent.
4. Cell fixation, cell washing and cell staining should be done on 96-well round bottom plates, 12 x 75 mm tubes, or 15- or 50-mL Falcon tubes. We do not recommend using 96-well flat bottom plates or 1.5 microcentrifuge tubes for cell fixation, cell washing or cell staining.

10. REFERENCES

1. Gupta RS. Cross-resistance of nocodazole-resistant mutants of CHO cells toward other microtubule inhibitors: Similar mode of action of benzimidazole cabamate derivatives and NSC 181928 and TN-16. *Mol Pharmacol.* 1986;30:142–148.
2. Ho J, Gillis K, Fishwild D. A simple and robust system for determining cell cycle distribution: The Guava cell cycle assay. Guava Application Note. 2004.

11. 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.

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Revision Date: November 14, 2005
4600-0790 rev B

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