



## Guava<sup>®</sup> Express CD3/CD4 Reagent Kit

Cat No. 4500-0150 100 Tests

### For Enumeration of CD3<sup>+</sup>CD4<sup>+</sup> T Lymphocytes in Blood

#### 1. PRODUCT DESCRIPTION AND INTENDED USE

The Guava<sup>®</sup> Express CD3/CD4 Kit is a two-color direct immunofluorescence reagent kit for enumeration of mature CD4<sup>+</sup> T lymphocytes in human blood. The kit consists of a monoclonal anti-human CD3 antibody conjugated to the tandem dye phycoerythrin (PE)-Cy5 (PECy5), a monoclonal anti-human CD4 antibody conjugated to PE, Guava 1X Lysing Solution to lyse erythrocytes and Guava Fixative to preserve the cells. The CD3 antibody uniquely identifies T cells and recognizes an epitope that is expressed on the epsilon chain of the CD3/T cell antigen receptor (TcR) complex. The CD4 antibody allows the identification of human helper/inducer CD4<sup>+</sup> T cell (HLA Class II reactive) and recognizes a 60,000 Da MW surface antigen. However, the CD4 antigen is also present on monocytes but at much lower density. Monocytes also lack expression of the CD3 molecule.

This product is for Research Use Only and is not intended for diagnostic use.

#### 2. MATERIALS PROVIDED

Guava Express CD3/CD4 Reagent Kit includes the following reagents:

##### **Guava Express CD3/CD4 Reagent Kit (Cat No. 4500-0150) 100 tests**

- Anti-Human CD4-PE Reagent (1 x 0.1 mL)
- Anti-Human CD3-PECy5 Reagent (1 x 0.1 mL)
- Guava Antibody Dilution Buffer (1 x 1 mL)
- Guava 1X Lysing Solution (1 x 20 mL)
- Guava Fixative (1 x 0.5 mL)

#### 3. HANDLING AND STORAGE

1. Store the Guava Express CD3/CD4 Antibody Reagents refrigerated (2 to 8°C). Do not freeze. Refer to the expiration date on the package label. Do not use the reagent after the expiration date.
2. The Guava Express CD3/CD4 Antibody Reagents contains light-sensitive dyes. Shield from excessive exposure to light.
3. Store the Guava Antibody Dilution Buffer refrigerated (2 to 8°C). Do not freeze. Refer to the expiration date on the package label. Do not use the reagent after the expiration date.
4. Store Guava 1X Lysing Solution at room temperature (18 to 25°C). Do not freeze or refrigerate. Refer to the expiration date on the package label. Do not use the reagent after the expiration date.
5. Store Guava Fixative at room temperature (18 to 25°C). Do not freeze or refrigerate. Refer to the expiration date on the package label. Do not use the reagent after the expiration date.

#### 4. WARNINGS AND PRECAUTIONS

1. The Guava Express CD3/CD4 kit is intended for research use only and not intended for diagnostic use.
2. The Guava Express CD3/CD4 reagents contain 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.
3. Guava Fixative contains formaldehyde. Formaldehyde is toxic, allergenic and a suspected carcinogen. Avoid ingestion, inhalation or contact with eyes, skin or clothing.

4. 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.
5. Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using these reagents.
6. Exercise standard precautions when obtaining, handling, and disposing of potentially carcinogenic and mutagenic reagents.
7. Exercise care to avoid cross contamination of samples during all steps of this procedure, as this may lead to erroneous results.
8. Dispose of all materials using proper precautions in accordance with federal, state and local regulations.
9. Deviations from the recommended procedure may adversely affect the overall results.
10. Material Safety Data Sheets for the reagents are available upon request.

## 5. EQUIPMENT AND MATERIALS REQUIRED

- Guava Technologies PCA instrument with EasyCD4 and Cytosoft™ software
- Guava Anti-Human CD4-PE Reagent
- Guava Anti-Human CD3-PE-Cy5 Reagent
- Guava Antibody Dilution Buffer
- Guava 1X Lysing Solution
- Guava Fixative
- Guava Check Beads (Catalog No. 4500-0020)
- K<sub>3</sub>EDTA (lavender top) Blood Sample for primary antibody labeling
- Pipettes
- Sample acquisition tubes, 1.5 mL microcentrifuge tubes with screw caps (VWR, Catalog No. 20170-215 or equivalent)
- Vortex Mixer
- Disposable pipette tips
- Disposable Gloves
- Guava Instrument Cleaning Fluid (ICF) (Catalog No. 4200-0140)
- Deionized, distilled or RO water
- 10% Bleach Solution

## 6. BEFORE YOU BEGIN

1. Turn on the laptop computer.
2. After the computer startup is completed, turn on the Guava PCA.
3. Start CytoSoft by double-clicking the **CytoSoft** application icon on the desktop.
4. When initialization is complete, wait for at least 15 minutes for laser warm-up before running acquisition. If you do a Clean & Shutdown and then go back to the main page of CytoSoft, the laser will not turn on. You must enter an application to turn on the laser.

## 7. REAGENTS AND SAMPLE PREPARATION

The following procedure is provided to allow the enumeration of CD4<sup>+</sup> lymphocytes in a peripheral blood preparation. An Antibody Working Solution and a Lyse/Fix Solution is prepared fresh daily by the user according to Table 1 and Table 2 respectively. Whole blood is first stained with the CD4 Antibody Working Solution, which binds to antigens on surface of lymphocytes; it is next treated with Guava Lyse/Fix Solution to lyse erythrocytes and preserve the cells and finally acquired on the Guava PCA to obtain the absolute CD4<sup>+</sup> counts. The blood used for the procedure may be collected by venipuncture into a sterile K<sub>3</sub>EDTA (lavender top) blood collection tube. Blood should be stained within 48 hours of collection for optimal results. Unstained anticoagulated blood should be maintained at 18 to 25°C prior to sample processing. Blood samples that are hemolyzed, clotted, lipemic, discolored or containing interfering substances should be discarded. The total processing time for one sample is about 35 minutes. Additional samples have a minimal effect on total staining time. Samples should be acquired within 3 hours of staining.

### CD3<sup>+</sup>CD4<sup>+</sup> Staining in Tubes

1. Determine the number of tests to be performed in a batch run. Prepare Guava Express CD3/CD4 Antibody Working Solution consisting of Guava anti-human CD4-PE Reagent, anti-human CD3-PE-Cy5 Reagent and Guava Antibody Dilution Buffer according to Table 1.
2. Prepare Guava Lyse/Fix Solution consisting of Guava 1X Lysing Solution and Guava Fixative according to Table 2. Prepare a little more Guava Express CD3/CD4 Antibody Working Solution and Guava Lyse/Fix Solution than you need for the number of patient samples to be acquired due to incomplete recovery of the total volume from the tube containing the working solution. Prepare the working solutions fresh each day of use.
3. Pipette 10 µL of Guava Express CD3/CD4 Antibody Working Solution to each labeled sample tube.
4. Pipette 10 µL of whole blood (from a well mixed EDTA lavender top tube) into the bottom of a 1.5 mL micro centrifuge tube. Cap the tube and then vortex each sample at medium intensity. Avoid leaving blood to dry on the side of the tube. This may cause erroneous results.
5. Incubate for 15 minutes at room temperature (18 to 25°C) in the dark.
6. Pipette 180 µL of Guava Lyse/Fix Solution into each tube to bring the total sample volume to 200 µL. Vortex on medium for 2-3 seconds and then incubate in the dark for 15 minutes at room temperature (18 to 25°C). Acquire samples within 3 hours of the lyse/fix preparation.
7. Samples are ready for acquisition and analysis on the Guava PCA system using the EasyCD4 software. Refer to the Guava User Guide for instructions on acquisition and analysis of the stained samples.
8. At the end of each batch run of stained samples, perform Quick Clean twice; first with ICF followed by 1:1 solution of ICF and bleach per the Guava User Guide. A Clean and Shutdown should be performed before the PCA is turned off for the day.

**Table 1. Preparation of Guava Express CD3/CD4 Antibody Working Solution**

Volume per tube (µL)	No. of tubes to be Prepared	Volume of Anti-Human CD4-PE Antibody Required (µL)	Volume of Anti-Human CD3-PECy5 Reagent Required (µL)	Volume of Guava Antibody Dilution Buffer Required (µL)	Total Volume (µL)
10	1	1	1	8	10
10	10	10	10	80	100
10	20	20	20	160	200
10	30	30	30	240	300
10	40	40	40	320	400
10	50	50	50	400	500

**Table 2. Preparation of Guava Lyse/Fix Solution**

Volume per tube (µL)	No. of tubes to be Prepared	Volume of Guava 1X Lysing Solution Required (µL)	Volume of Guava Fixative Required (µL)	Total Volume Prepared (µL)	Total Volume Required (µL)
180	1	195	5	200	180
180	10	1950	50	2000	1800
180	20	3900	100	4000	3600
180	30	5850	150	6000	5400
180	40	7800	200	8000	7200
180	50	9250	250	9500	9000

## 9. EXPECTED RESULTS

The Guava Express CD3/CD4 application performs calculations automatically. The results are displayed on the computer screen after each sample is acquired. Acquired data are displayed in a dot plot with a gate. Movable cursors allow the location and size of the gate to be altered. The analysis results of each sample are automatically displayed at the end of each acquisition and include the mean fluorescence intensities of the sample along with the counts and percentage of events falling within the CD3 gate. Also displayed is the absolute CD4 count that is obtained by dividing the number of CD3<sup>+</sup>CD4<sup>+</sup> cells by the total volume in which counts are acquired, and multiplying by the dilution factor. The resulting value is the absolute CD4 counts/ $\mu$ l blood for the sample. All data and instrument settings are automatically saved to spreadsheet files for future reference. The data can be reanalyzed later using CytoAnalysis. If you selected the option to save data in FCS 2.0 file format, you can analyze the data with third party FCS 2.0-compatible programs.

An example of staining results obtained using the Guava Express CD3/CD4 Kit is shown in Figure 1 and Figure 2. You may opt to analyze the CD4<sup>+</sup> population by using either quadrant markers (Figure 1) or by rectangular gate (Figure 2). The CD3<sup>+</sup>CD4<sup>+</sup> cells are shown in magenta in upper right quadrant of Figure 1 and the center gate in Figure 2. The CD3<sup>+</sup>CD4<sup>-</sup> cells (mostly CD3<sup>+</sup>CD8<sup>+</sup>) are to the left of the CD3<sup>+</sup>CD4<sup>+</sup> T cells. Monocytes are below the CD4<sup>+</sup> T lymphocytes and are characterized by being CD3 negative and having dim CD4<sup>+</sup> expression.

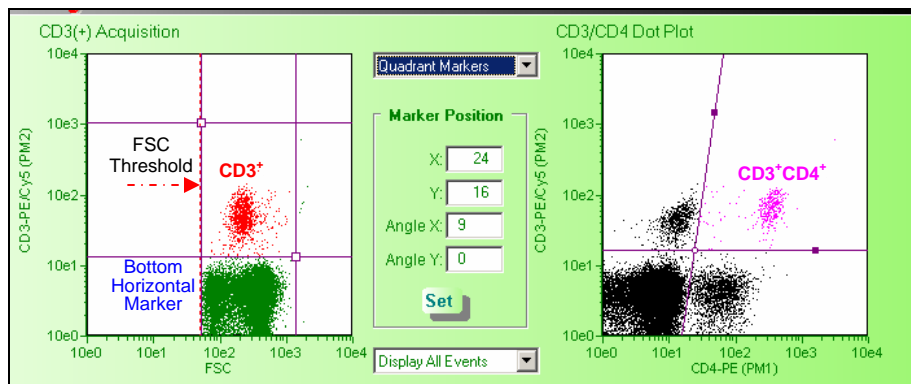


Figure 1.

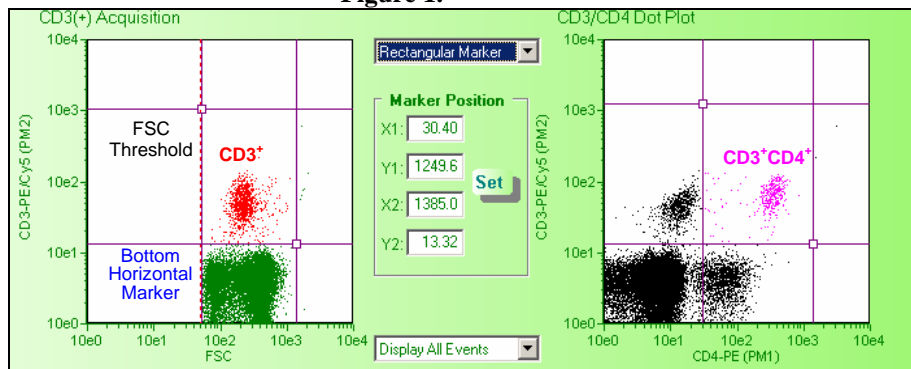


Figure 2.

## 10. TROUBLESHOOTING TIPS

1. Mix each sample thoroughly on a vortex mixer before acquiring samples for consistent and accurate results.
2. If the concentration of the stained cell sample for data acquisition is high ( $> 6 \times 10^5$  cells/mL), the Guava PCA may not yield accurate results. Dilute the sample further with additional Guava

- Lyse/Fix Solution to bring the cell concentration into an acceptable range and make any adjustments necessary to the dilution factor on the CytoSoft acquisition screen.
3. Run the Guava Check application using the Guava Check kit (Catalog No. 4500-0020) each day of use, prior to using the Guava PCA, to verify proper instrument function and accuracy.
  4. Periodically run Quick Clean using a deionized water tube (after every 15 to 20 sample acquisitions) to prevent a buildup from cell debris in the flow system. If your samples contain significant amounts of cellular debris, run Quick Clean more often to prevent clogs or blockage. Quick Cleans may also be run with ICF. Be sure to follow with a Quick Clean of deionized water.
  6. Cell aggregates, cell debris, bleach crystals, or other particulates can cause a clog or blockage of the flow system. 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 a blockage of the flow system. Change the sample tube with a 20% bleach tube. Click **Backflush** to flush out the clog. Load a deionized water tube and run Quick Clean to remove bleach residue. If this procedure does not alleviate the problem, consult the *Guava PCA User Guide* or contact Technical Service for additional help. For more troubleshooting tips, refer to the *Guava PCA User Guide*.
  7. Remove any dried blood from the sides of the tube prior to vortexing with reagent. Unstained blood will contribute to erroneous results.
  8. Samples should be acquired within 3 hours of addition of the Guava Lyse/Fix Solution. It is recommended that the Guava Lyse/Fix Solution be added in batches of 10 to 20 samples at a time to avoid over incubation.
  9. Samples with excessive amounts of debris may cause the software to pause for extended periods. Allow the software to recover for 30 to 60 seconds before terminating the run.
  10. It is recommended that approximately 5 mL of bleach be added to the waste vial prior to the first use of the PCA and every time after the waste vial is emptied.

## 11. LIMITATIONS

The Guava PCA will yield optimal results when the stained cell sample for data acquisition is between  $1 \times 10^4$  to  $6 \times 10^5$  cells/mL. To obtain the most accurate assay results, adjust the concentration of the cell samples to within the recommended range.

## 12. 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.

## 13. RETURN GOODS POLICY

Please inspect package(s) upon receipt and inform us immediately of any shortage or shipping errors. Claims must be made within 10 business days. Call our Customer Service department so they can authorize a return and provide shipping instructions.

## 14. TRADEMARKS AND PATENTS

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To order the Guava PCA call (510) 576-1400 or visit [www.guavatechnologies.com](http://www.guavatechnologies.com).

For more information, email: [support@guavatechnologies.com](mailto:support@guavatechnologies.com)



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