



Guava® Auto CD4/CD4% Kit

Catalog No. 4500-0480 100 Tests

Catalog No. 4500-0485 500 Tests

Catalog No. 4500-0490 1000 Tests

**For Enumeration of CD4+
T Lymphocytes in Blood and
Determination of CD4+ T-cells as a
Percent of Total Lymphocytes**

The Guava Auto CD4/CD4% Kit and components are not for diagnostic use in the United States, European Union, Japan, and certain other regulated countries.

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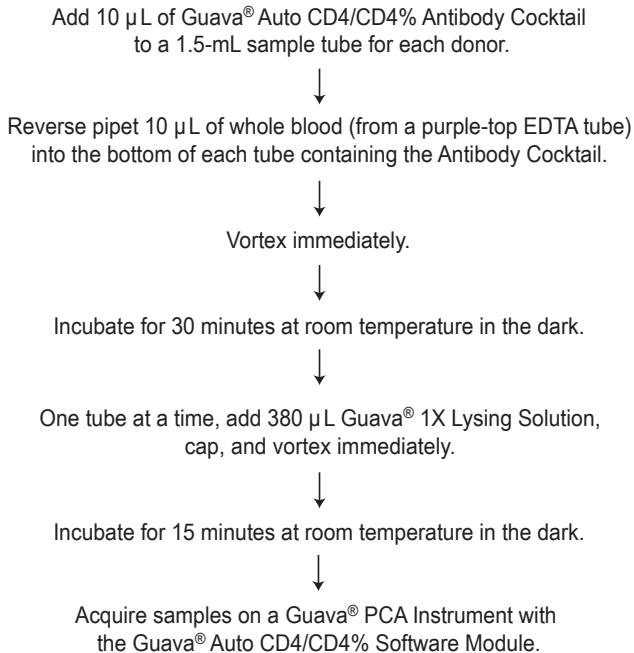
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I Introduction

Overview

The Guava[®] Auto CD4/CD4% Kit is a two-color direct immunofluorescence reagent kit for enumeration of mature CD4+ T lymphocytes and the determination of CD4 T cells as a percent of total lymphocytes in human blood. The kit consists of the antibody cocktail, a proprietary mixture of anti-human lymphocyte antibodies conjugated to the tandem dye phycoerythrin (PE)-Cy5 (PECy5) and a monoclonal anti-human CD4 antibody conjugated to PE, and Guava[®] 1X Lysing Solution to lyse erythrocytes. The anti-human lymphocyte antibodies detect all human lymphocytes. The CD4 antibody allows the identification of human helper/inducer CD4+ T cell (HLA Class II reactive) and recognizes a 60,000 Da surface antigen. Monocytes also express CD4 but at lower density, and have no co-expression of the other lymphocyte markers detected by reagents in this kit. The Auto CD4/CD4% Kit should be used only with the Guava[®] Auto CD4/CD4% Software Module, which allows for automatic acquisition and gating and provides test results.

Guava Auto CD4/CD4% Assay Workflow



Safety

1. Wear proper laboratory attire (lab coat, gloves, safety glasses) when handling or using these reagents.
2. All biological specimens and materials should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Avoid specimen contact with skin and mucous membranes.
3. The Auto CD4/CD4% Kit components contain sodium azide, which is toxic. Contact with acids liberates toxic gas. Dispose of all materials according to federal, state or local regulations.
4. Guava Technologies recommends that approximately 5 mL of bleach be added to the waste vial prior to using the Guava[®] PCA Instrument.
5. The MSDS for the reagents are available from our web site (www.guavatechnologies.com), or by contacting Guava Technologies Technical Support (support@guavatechnologies.com; telephone 866-448-2827 ext 1428 (USA only), or 510-576-1428).

Required Materials

Handling and Storage

1. Store the Auto CD4/CD4% Antibody Cocktail 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. Return the Auto CD4/CD4% Antibody Cocktail to 2 to 8°C immediately after use. Do not leave out at higher temperatures for extended periods of time.
3. The Auto CD4/CD4% Antibody Cocktail contains light-sensitive dyes. Shield from excessive exposure to light.
4. Store the 1X Lysing Solution at room temperature (18 to 25°C). Do not freeze. Refer to the expiration date on the package label. Do not use the reagent after the expiration date.
5. During shipment, small volumes of product can become entrapped in the lid of the product vial. For complete recovery, briefly centrifuge the vial of Guava Auto CD4/CD4% Antibody Cocktail in a tabletop centrifuge prior to opening. Alternatively use a pipet tip to recover material entrapped in the cap.
6. Avoid microbial contamination of reagents or erroneous results may occur.

Kit Materials

Guava Auto CD4/CD4% Kit includes one of the following:

Reagent Kit Part Number	Contents
4500-0480 Guava Auto CD4/CD4% Kit 100 Tests	One 1-mL vial of Guava Auto CD4/CD4% Antibody Cocktail (Part Number 4700-1160) One 40-mL bottle of Guava 1X Lysing Solution (Part Number 4700-0082)
4500-0485 Guava Auto CD4/CD4% Kit 500 Tests	Five 1-mL vials of Guava Auto CD4/CD4% Antibody Cocktail (Part Number 4700-1160) Five 40-mL bottles of Guava 1X Lysing Solution (Part Number 4700-0082)
4500-0490 Guava Auto CD4/CD4% Kit 1000 Tests	Ten 1-mL vials of Guava Auto CD4/CD4% Antibody Cocktail (Part Number 4700-1160) Ten 40-mL bottles of Guava 1X Lysing Solution (Part Number 4700-0082).

User Supplied Equipment and Materials

- Guava[®] PCA Instrument with Guava CytoSoft™ Software and Auto CD4/CD4% Software Module
- Guava[®] Check Kit (Catalog No. 4500-0020)
- EDTA-whole blood, less than 48 hours since collection
- Pipettes, calibrated to ensure accuracy
- Sample acquisition tubes, 1.5 mL microcentrifuge tubes with screw caps (VWR Catalog No. 16466-064 or equivalent)
- Vortex mixer
- Disposable pipette tips
- Disposable gloves
- Guava[®] Instrument Cleaning Fluid (ICF) (Catalog No. 4200-0140)
- Deionized, distilled, or RO water
- 20% bleach solution

II Protocol

Specimen Collection and Preparation

The blood used for the procedure should be collected by venipuncture into a sterile K3 EDTA (lavender top) blood collection tube.

WARNING: *Blood samples that are hemolyzed, clotted, lipemic, discolored, or containing interfering substances should be discarded.*

NOTE: Blood should be stained within 48 hours of collection for optimal results. Unstained anticoagulated blood should be maintained at 18-25°C prior to sample processing.

NOTE: Leave the capped tubes of blood standing upright or lying on their sides if it is stored overnight. Do not rock or agitate blood in any way during extended storage.

Instrument Set-Up

1. Always perform a Guava Check Procedure on the Guava PCA Instrument using the protocol detailed in the Guava Check Kit package insert, prior to acquiring prepared samples.

NOTE: It is necessary for the Guava Check Procedure results to pass for all criteria before continuing.

- a. The bead count must be within 10% of the Lot Specific Bead Count, which is found on the information card that accompanies the Guava Check Kit.
- b. The % CV for bead count, and for FSC, PM1 and PM2 intensities should be <10%.
- c. Other parameters to monitor include: FSC intensity (>150), PM1 mean fluorescence intensity (between 765-1320) and PM2 mean fluorescence intensity (between 171-275).

NOTE: More information can be found in the *Guava Auto CD4/CD4% System User's Guide*.

2. Perform a Quick Clean cleaning procedure after the Guava Check Procedure and prior to acquiring the samples prepared with the Auto CD4/CD4% Kit.

Staining Samples and/or Controls

1. Label one 1.5 mL tube per donor with sample identification information such as donor number.
2. Pipette 10 µL of Auto CD4/CD4% Antibody Cocktail into each 1.5-mL microcentrifuge sample tube for each donor.

CAUTION: Put the stock bottle of Auto CD4/CD4% Antibody Cocktail back into the refrigerator or on ice immediately after use. Do not allow the bottle of Auto CD4/CD4% Antibody Cocktail to remain at elevated temperatures for extended times.

3. Reverse pipet 10 μL of whole blood (from a well mixed EDTA lavender top tube) into the bottom of each tube containing the Antibody Cocktail.

NOTE: Blood in the tubes should be thoroughly resuspended by gentle agitation for a few minutes before removing an aliquot for sample preparation.

4. Cap the tubes and then vortex each sample immediately at medium intensity for 3–5 seconds.

CAUTION: Avoid leaving blood to dry on the side of the tube. This may cause erroneous results.

5. Incubate for 30 minutes at room temperature (18 to 25°C) in the dark.
6. Remove tubes from the dark.
7. Pipette 380 μL of 1X Lysing Solution directly into one of the tubes to bring total sample volume to 400 μL .
8. Vortex that tube immediately on medium intensity for 3–5 seconds.
9. Repeat the addition of 1X Lysing Solution and vortexing for each of the remaining tubes.
10. Incubate the samples for 15 minutes at room temperature (18 to 25°C) in the dark.
11. Samples are ready for acquisition and analysis on the Guava PCA Instrument using the Auto CD4/CD4% Software Module.

NOTE: Batch your preparations to avoid over-incubation of samples. Samples must be acquired within 4 hours after preparation.

Sample Acquisition

NOTE: Sample acquisition and analysis details are found in the *Guava Auto CD4/CD4% System User's Guide*.

1. Acquire each sample on the Guava PCA Instrument.

NOTE: Vortex each prepared sample thoroughly before loading it on the Guava PCA Instrument for data acquisition.

2. When finished acquiring samples run a Clean and Shut Down as described in the *Guava Auto CD4/CD4% System User's Guide*.

Expected Results

The Guava Auto CD4/CD4% Software Module adjusts settings, acquires samples and provides test results automatically. The results are displayed on the computer screen after each sample is acquired. Results displayed are dependent upon whether the software is used in operator mode or supervisor mode.

Results displayed in operator mode (shown in Figure 1) include sample information, and test results for CD4 T Cells in original sample (cells/ μ L), CD4% of Lymphocytes, and Total Lymphocytes in original sample (cells/ μ L).

The screenshot displays the software interface with the following data:

Field	Value
Reagent Kit Lot #	102407
Reagent Kit Expiration Date	29 Feb 2008
Test Operator	Jan Adams
Lab Director	Aaron Jones, MD
Test Site	Blake Central Laboratory
Donor ID	3942039
Date & Time of Blood Draw	29 Nov 2007 20:24:57
Original Sample Values	CD4 T Cells = 2400.2 cells/ μ L CD4% = 61.8 Total Lymphocytes = 3882.2 cells/ μ L
Analysis Operations	Open Data Set

Figure 1. Test results for an adult blood sample displayed in operator mode.

The software also displays error messages when it is unable to run or gate the samples as shown in Figure 2.

The screenshot displays the software interface with the following data:

Field	Value
Reagent Kit Lot #	102407
Reagent Kit Expiration Date	23 Apr 2008
Test Operator	The Operator
Lab Director	The Director
Test Site	Guava Technologies
Donor ID	D# 2
Date & Time of Blood Draw	15 Nov 2007 9:08:31
Original Sample Values	Error <i>A01: The sample could not be autogated.</i> <i>W01: The sample has low Total Lymphocytes.</i>
Analysis Operations	Open Data Set Preview Sample Report Preview Data Set Report

Figure 2. Test results for a blood sample displaying error messages in operator mode.

NOTE: Results displayed in the supervisor mode provide additional functionality, which includes visualization of a dot-plot showing the staining pattern for the sample, sample information, as well as test results for CD4 T Cells in original sample (cells/ μ L), CD4% of Lymphocytes and Total Lymphocytes in original sample (cells/ μ L). There is also an option for a supervisor to change the automatic gating done by the software in the Supervisor Mode by unchecking the Automated Gating button and moving markers to obtain manual gating results.

The individual sample results may be printed using the Print Sample Report button on the Analysis screen. The Sample Report can be viewed or printed in operator or supervisor mode for each individual sample using the Preview or Print Sample Report button found on the Analysis screen. The Sample Report contains sample ID, CD4 T Cell counts, Total Lymphocyte counts, and CD4% information. The Sample Report also indicates if a sample was gated automatically or manually. Any errors or warnings that occurred are also displayed in the report.

A summary report for each data set can be generated in operator or supervisor mode by clicking on the Preview Data Set Report button or printed by clicking on the Print Data Set Report button. An example of the Data Set Summary Report is shown in Figure 3.

Guava® Auto CD4/CD4% Data Set Summary Report													
Current Date - 22 JAN 2008											10:06		
File name - Dataset1208_AAP-FCS													
Instrument Serial Number - GT10300418													
Software version number - Auto CD4/CD4% Version 6.1fc8													
Test Site -													
Lab Director -													
Sample Summary								Tracking Information					
Sample No.	Sample ID	Donor ID	CD4 T cell Conc. in donor sample (cells/ μ L)	CD4% in donor sample	Total Lymphocyte Conc. in donor sample (cells/ μ L)	Error or Warning code	Auto or Manual gating	Reagent Lot #	Reagent Expiration Date	Date of Blood Draw	Sample Start Date	Sample Start Time	Test Operator
1	G1		258.3	9.8	2641.1		Auto	091307	16 MAR 2008		17 DEC 2007	17:03:14	The Operator
2	G2		881.8	30.6	2881.4		Auto	091307	16 MAR 2008		17 DEC 2007	17:05:25	The Operator
3	G3		269.5	11.1	2432.1		Auto	091307	16 MAR 2008		17 DEC 2007	17:07:47	The Operator
4	G4		297.1	14.8	2012.9		Auto	091307	16 MAR 2008		17 DEC 2007	17:10:23	The Operator
5	G5		556.5	26.7	2083.6		Auto	091307	16 MAR 2008		17 DEC 2007	17:13:11	The Operator
6	G6					A01, W01	Auto	091307	16 MAR 2008		17 DEC 2007	17:18:11	The Operator
7	G7		367.4	13.2	2778.2		Auto	091307	16 MAR 2008		17 DEC 2007	17:21:11	The Operator
8	G8		206.6	10.2	2022.6		Auto	091307	16 MAR 2008		17 DEC 2007	17:23:50	The Operator
9	G9		345.8	7.9	4373.2		Auto	091307	16 MAR 2008		17 DEC 2007	17:25:20	The Operator
10	G10		378.3	16.4	2302.5		Auto	091307	16 MAR 2008		17 DEC 2007	17:27:40	The Operator
11	G11		456.5	27.9	1636.3		Auto	091307	16 MAR 2008		17 DEC 2007	17:30:44	The Operator
12	G12		238.9	15.9	1505.5		Auto	091307	16 MAR 2008		17 DEC 2007	17:34:05	The Operator
13	G13		386.3	11.5	3374.0		Auto	091307	16 MAR 2008		17 DEC 2007	17:35:48	The Operator
14	G14		405.4	17.2	2354.7		Auto	091307	16 MAR 2008		17 DEC 2007	17:38:04	The Operator
15	G15					A01	Auto	091307	16 MAR 2008		17 DEC 2007	17:41:00	The Operator
16	G16		151.5	8.5	1789.6		Auto	091307	16 MAR 2008		17 DEC 2007	17:43:46	The Operator
17	G17		573.7	31.8	1804.2		Auto	091307	16 MAR 2008		17 DEC 2007	17:46:31	The Operator
18	G18		290.0	10.8	2680.3		Auto	091307	16 MAR 2008		17 DEC 2007	17:48:37	The Operator

#W01: The sample has low Total Lymphocytes. #W02: The sample may not be correctly autogated. #W03: After acquisition, CD4 outside linear range.
 #W04: After acquisition, CD4% outside linear range. #E02: User aborted run. #E03: Run aborted. Too few cells detected. #E04: After acquisition, too few lymphocytes.
 #E06: Run aborted. Sample too concentrated. #E07: After acquisition, sample too concentrated. #E08: Pump error. Data not saved.
 #E09: After acquisition, too few lymphocytes, gated manually. #E10: After acquisition, sample too concentrated, gated manually.
 #E11: Loader Arm is in down position. The run has been aborted. #A01: The sample could not be autogated.

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Figure 3. Summary report for an entire data set.

IV Troubleshooting and Limitations

Troubleshooting Tips

1. If results are not accurate or consistent, check staining, count and % results from a control sample(s) using normal adult blood or a commercially available whole blood control(s) following the above protocol to assess proper functioning of the assay, software and instrument. It is good practice to run such controls once a day. For a list of whole blood controls that work with the Auto CD4/CD4% Assay please contact Guava Technologies.
2. If the results are not accurate or consistent, check that each sample was thoroughly mixed before acquiring.
3. If there appears to be day-to-day variation of the results, ensure that the Guava Check Procedure using the Guava Check Kit (Catalog No. 4500-0020) was run each day and passed for counts and intensities prior to using the Guava PCA Instrument to acquire prepared blood samples.
4. If you notice inaccurate results from samples, be sure that any dried blood from the sides of the sample preparations tubes was removed prior to vortexing the blood with the Auto CD4/CD4% Antibody Cocktail. Unstained blood will contribute to erroneous results.
5. If the software reports error message “E06: Run aborted. Sample too concentrated,” or “E07: After acquisition, sample too concentrated,” or “E10: After acquisition, sample too concentrated, gated manually,” it may imply that the prepared sample is too concentrated, has >500 lymphocytes/ μL , and needs to be diluted. If you see such an error message prepare samples again according to the procedure above; however in step 7 of the Procedure add $580 \mu\text{L}$ of 1X Lysing Solution (instead of $380 \mu\text{L}$) to the sample tube, lyse for 15 minutes and acquire the sample on the Guava PCA Instrument. At the Acquisition screen, be sure to change the dilution factor to 60 before acquisition.
6. If the software reports error message “W02: The sample may not be correctly autogated” or “A01: The sample could not be autogated,” proceed to the supervisor mode and view dot-plots to determine if manual gating of the samples is possible. Not staining or lysing for recommended times might give rise to poor staining patterns that cannot be autogated. Blood not stored at recommended temperatures can deteriorate and when used in the assay give rise to poor staining patterns.
7. If you notice that results appear to be changing over time, the prepared samples may be too old. Once prepared, samples are stable for 4 hours.
8. If you notice carryover between samples, the flow cell capillary may be rinsed between each sample by loading a tube containing deionized water, and then unloading it before loading each prepared sample tube.

9. If you are acquiring data from a sample but the progress bar is not moving, or if you received an error message stating you might have a clog, there is probably a blockage of the flow system. Cell aggregates, cell debris, salt crystals, or other particulates can cause a clog or blockage of the flow system. Change the sample tube to one containing 20% bleach. Click Backflush to flush out the clog. Load a deionized water tube and run a Quick Clean cleaning cycle to remove bleach residue. If this procedure does not alleviate the problem, consult the *Guava Auto CD4/CD4% System User's Guide* or contact Technical Support for additional help.
10. If your instrument clogs frequently, your samples may contain significant amounts of cellular aggregates and debris that might build up in the flow system. Periodically run a Quick Clean cleaning cycle using a deionized water tube (after every 15 to 20 sample acquisitions). Quick Clean cleaning may also be run with ICF. In that case, be sure to follow with a Quick Clean cleaning cycle using deionized water.
11. If you notice the software pausing for extended periods, the sample may contain excessive amounts of debris. Allow the software to recover for 30–60 seconds before terminating the run.
12. If error messages are obtained on the results screen consult the *Guava Auto CD4/CD4% System User's Guide* for a description of errors and troubleshooting to be performed.

For more troubleshooting tips, refer to the *Guava Auto CD4/CD4% System User's Guide* or contact Guava Technologies Technical Support.

Limitations

1. The results are dependent upon proper handling of samples, reagents and instruments as instructed in this package insert.
2. The results are guaranteed only for use of the Auto CD4/CD4% Kit along with the Auto CD4/CD4% Software Module when used on the Guava PCA Instrument platform.
3. Accurate results will be obtained only if the Guava Auto CD4/CD4% System has passed the Guava Check Procedure and all parameters including Particles/mL (mean and %CV), FSC intensity, PM1 MFI and PM2 MFI values have passed.
4. The Guava Auto CD4/CD4% Assay has not been validated for use with whole blood containing either heparin or acid citrate dextrose (ACD) liquid anticoagulants in determining absolute counts.
5. The blood specimens must be stored in blood collection tubes at room temperature for <48 hours for use in the assay.

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.

Returned 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.

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For ordering information or technical support, contact your local distributor or the nearest office listed below:

U.S. and Canada

Phone: 1-866-448-2827 ext. 1428 (USA only), or 1-510-576-1428

Fax: 1-510-576-1500

support@guavatechnologies.com

Africa

Phone: +44(0)1780 764390

Fax: +44(0)1780 765322

africaservice@guavatechnologies.com

Europe

Phone: +44(0)1780 764390

Fax: +44(0)1780 765322

euroservice@guavatechnologies.com

Asia and Pacific

Phone: +91 124 4148247

Fax: +91 124 4148504

asiaservice@guavatechnologies.com

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Hayward, CA 94545-2991
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