BD FACSCount™ CD4

For enumerating absolute counts and determining percentages of CD4 T lymphocytes in unlysed whole blood

50 Tests—Catalog No. 339010

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1. INTENDED USE

BD FACSCount™ CD4 reagents are used to enumerate the absolute counts of CD4 T lymphocytes and determine the percentage of lymphocytes that are CD4 T lymphocytes in unlysed whole blood (CD4 counts and CD4 percentages). The reagents are intended for in vitro diagnostic use on a BD FACSCount™ instrument.

Clinical Applications

CD4 counts and CD4 percentages have been used to evaluate the immune status of patients with, or suspected of developing, immune deficiencies such as acquired immune deficiency syndrome (AIDS).[1,2]

The CD4 antigen is the receptor for the human immunodeficiency virus (HIV).[3] The absolute number and percentage of CD4 T lymphocytes are the cellular parameters most closely associated with HIV disease progression and patient prognosis.[4] The number of CD4 T lymphocytes declines in HIV infection.[5-7]

2. PRINCIPLES OF THE PROCEDURE

A single test requires one ready-to-use reagent tube.

When whole blood is added to the reagent tube, fluorochrome-labeled antibodies in the reagents bind specifically to white blood cell surface antigens, and a fluorescent nuclear dye binds to the nucleated blood cells. After a fixative solution is added, the sample is run on the instrument. During sample acquisition, the cells pass through the laser light, which causes the labeled cells to fluoresce. This fluorescent light provides the information necessary for the instrument

to identify and count the lymphocytes and CD4 T lymphocytes.

In addition, the reagent tubes contain a known number of fluorescent reference beads to which a precise volume of whole blood is added. The software automatically identifies the lymphocyte populations of interest and calculates the CD4 counts (cells/μL) by comparing cellular events to bead events. Results include CD4 counts and CD4 percentages.

3. REAGENTS

Reagents Provided, Sufficient for 50 Tests

The following are provided:

- 50 reagent tubes containing CD4 PE/CD14 PE-Cy™5*/CD15 PE-Cy5, fluorescent nuclear dye, and reference beads
- 65 reagent tube caps

  NOTE Use the caps to prevent spillage of patient samples and controls while vortexing, during incubation, and before and after running samples on the instrument.
- One 5-mL vial of 5% formaldehyde in phosphate-buffered saline (PBS), used as fixative solution

The CD4 antigen,[8,9] 55 kilodaltons (kDa),[10] is present on a T-lymphocyte subset[11,12] that comprises 28% to 58%[13] of normal peripheral blood lymphocytes.[9,10] The CD4 antigen is present in low density on the cell surface of monocytes and in the cytoplasm of monocytes.

CD4, clone SK3,[8] is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immununized with human peripheral blood T lymphocytes. CD4 is composed of mouse IgG_1 heavy chains and kappa light chains.

CD14 recognizes a human monocyte/macrophage antigen, with a molecular weight of 55 kDa.[14] The CD14 antigen is present on the majority of normal peripheral blood monocytes.[15]

CD14, clone MϕP9, is derived from the hybridization of mouse Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with peripheral blood monocytes from a patient with rheumatoid arthritis. CD14 is composed of mouse IgG_{2b} heavy chains and kappa light chains.

CD15 recognizes a human myelomonocytic antigen.[16] The structure recognized by CD15 antibodies is lacto-N-fucopentose III.[16] The CD15 antigen is present on the majority of mature peripheral blood eosinophils and neutrophils and is present at low density on circulating monocytes.

CD15, clone MMA, is derived from the hybridization of mouse P3-X63-Ag8.653 myeloma cells with spleen cells from BALB/c mice immunized with the U-937 histiocytic cell line. CD15 is composed of mouse IgM heavy chains and kappa light chains.

The nuclear dye binds to nucleic acid and fluoresces.

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Concentration values are listed in the following table:

Reagent	Concentration
Beads	1.29 x 10^5 beads/mL
CD4	0.1 µg/mL
CD15	0.625 µg/mL
CD14	0.625 µg/mL
Oxazine	4.3 µg/mL

Precautions

- For In Vitro Diagnostic Use.
- The antibody reagents contain sodium azide as a preservative; however, care should be taken to avoid microbial contamination, which could cause erroneous results.

Fixative contains 5.0% formaldehyde, CAS number 50-00-0 and 1.76% methanol, CAS number 67-56-1.

	Danger
	H331 Toxic if inhaled.
	H341 Suspected of causing genetic defects. H350 May cause cancer. Route of exposure: Inhalative.
	H318 Causes serious eye damage.
	H302 Harmful if swallowed. H312 Harmful in contact with skin. H315 Causes skin irritation. H317 May cause an allergic skin reaction. H335 May cause respiratory irritation.
	Wear protective gloves / eye protection.Wear protective clothing. Avoid breathing mist/vapours/spray. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell.

WARNING The reagent solution contains a nuclear dye. The toxicological properties of this dye have not been investigated. If inhaled or ingested, contact a physician immediately. If skin or eye contact occurs, wash with copious amounts of water.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[17,18] and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing, eyewear, and gloves.

Storage and Handling

The reagent is stable until the expiration date shown on the label when stored at 2°C–8°C. Do not use after the expiration date. Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the outside of the reagent vial dry.

Do not use the reagent if you observe any change in appearance. Precipitation or discoloration indicates instability or deterioration.

Reagents or Materials Required but Not Provided

- BD Vacutainer® EDTA blood collection tubes or equivalent

- Disposable pipet tips (Catalog No. 340292) or equivalent
- Vortex mixer (See Recommended Brands of Materials in the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents*.)
- BD FACSFlow™ sheath fluid (Catalog No. 342003) or equivalent
- BD FACSCount™ controls (Catalog No. 340166)
- BD FACSCount system

4. INSTRUMENT

BD FACSCount CD4 reagents are designed for use on a BD FACSCount instrument. We recommend running BD FACSCount controls daily. Be sure to use the BD FACSCount CD4 protocol disk with the most recent control data when running samples stained with CD4 reagents on the BD FACSCount instrument. See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed instructions.

5. PROCEDURE

Collecting Blood

Collect blood aseptically by venipuncture, using EDTA blood collection tubes.[19] A minimum of 100 µL of whole blood is required for this procedure. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected to ensure proper specimen dilution, especially when determining absolute counts with reference beads.

Anticoagulated blood stored at room temperature (20°C–25°C) must be stained within 24 hours of draw and must be analyzed within 48 hours of staining.

NOTE Do not use previously fixed and stored patient samples. Whole blood samples refrigerated before staining can give aberrant results. Specimens obtained from patients taking immunosuppressive drugs can yield poor resolution.[20] Blast cells can interfere with test results. Hemolyzed specimens should not be used.

Performing Quality Control

We recommend performing a control run using BD FACSCount controls to check system accuracy and linearity. Run controls each day before you run patient samples or whenever you open a new reagent lot. See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed information on performing a BD FACSCount control run.

Preparing Tubes

NOTE We recommend that you prepare no more than 15 reagent tubes at one time.

1. Label the tab of each reagent tube with the patient accession number or number that identifies the tube of blood.
2. Vortex each tube upside down for 6 seconds and upright for 6 seconds.

   NOTE Set the vortex speed to a setting that causes the liquid to rise to the top of the tube.
3. Open each reagent tube with the coring station.

Adding Blood

1. Invert the EDTA tube 5 to 10 times to make sure that the whole blood is adequately mixed.

2. Pipette 50 µL of whole blood into the reagent tube labeled with the corresponding patient accession number.

   Reverse pipetting is critical to accuracy. We recommend using the BD FACSCount pipet that is provided with the BD FACSCount system.

   Pipette whole blood onto the side of the tube just above the liquid reagent.

   If an electronic pipet is not available, follow these instructions for manual reverse pipetting.

   - Depress the button to the second stop. When you release the button, excess sample is drawn up into the tip.
   - Depress the button to the first stop to expel a precise volume of blood. This leaves excess blood in the tip.

   Always change to a new tip between tubes. Discard tips in an appropriate biohazard container.

3. Cap the tube and vortex upright for 6 seconds.
4. Repeat steps 1 through 3 to prepare a sample tube for each patient specimen.
5. Incubate the tubes for 30 minutes at room temperature (20°C–25°C) in the workstation. Close the cover to protect the reagents from light.

   NOTE Correct incubation time is critical and must be at least 30 minutes but no longer than 40 minutes for each sample tube.

Adding Fixative

1. Uncap each sample tube and pipette 50 µL of fixative solution into each tube.

   Always change to a new tip between tubes. Discard tips in an appropriate biohazard container.

2. Recap each tube and vortex upright for 6 seconds.

Run the sample tubes on the BD FACSCount instrument within 48 hours of adding fixative. Store samples at room temperature, protected from light, until they are run on the instrument.

Running Patient Samples

See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for detailed information on running patient samples.

Make sure you enter the patient accession number in the software before you begin.

1. Vortex the CD4 tube upright for 6 seconds.

   WARNING Inadequate suspension of white blood cells can result in inaccurate results.

2. Uncap the tube and set the cap aside.
3. Place the sample tube in the sample holder and press Run.

   A software message will indicate when the analysis is complete.

4. Remove the sample tube and recap it.

   Discard the sample tube in an appropriate biohazard container.

5. Repeat steps 1 through 4 for the remaining samples.

6. EXPECTED RESULTS

Reference Ranges

The reference ranges for BD FACSCount CD4 reagents shown in Table 1 were determined at BD Biosciences in San Jose,

CA. Subjects were healthy adults between the ages of 18 and 65 years.

Table 1 Representative reference ranges for BD FACSCount CD4 reagents

Parameters	n[a]	Mean	95% Reference Range
Absolute CD4 (cells/µL)	141	906.65	380–1,704
Percent CD4	141	44.90	30.13–60.23

a. n=sample size

7. PERFORMANCE CHARACTERISTICS

Performance of the reagents was established by testing at BD Biosciences in San Jose, CA and at three clinical laboratories in the US.

Accuracy (Agreement)

CD4 absolute counts were enumerated and percentages were determined with BD FACSCount CD4 reagents on the BD FACSCount instrument using BD FACSCount CD4 software v1.0. Results were compared with results from the BD Tritest™ CD3 FITC/CD4 PE/CD45 PerCP reagent in BD Trucount™ tubes on the BD FACSCalibur™ flow cytometer using BD Multiset™ software.

Whole blood samples were collected at random at three clinical laboratories. Regression statistics are reported in Table 2.

Table 2 Regression analysis of test versus predicate for CD4 absolute counts and percentages

Parameters	n	R^2	Slope	Intercept	Range
Absolute CD4 (cells/µL)	101	0.981	0.971	12.695	59–3,405
Percent CD4	99	0.99	0.999	–0.391	5.51–64.69

Figure 1 Regression plot of test versus predicate for CD4 absolute counts (x-axis = BD FACSCount CD4 Absolute Counts, y-axis = BD Tritest CD4 Absolute Counts)

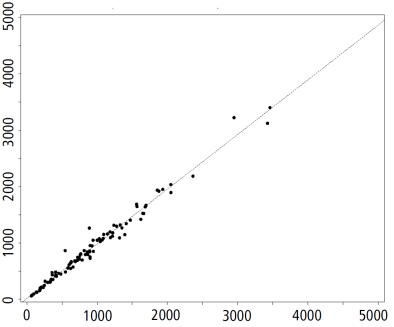
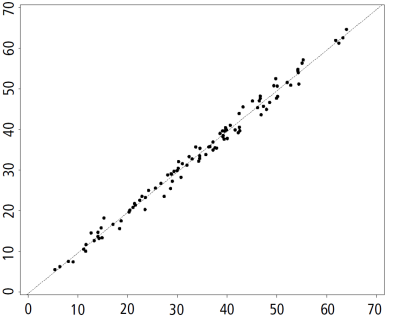


Figure 2 Regression plot of test versus predicate for CD4 percentages (x-axis = BD FACSCount CD4 Percentages, y-axis = BD Tritest CD4 Percentages)



Precision

Estimates of precision were determined at one site, BD Biosciences, using BD Multicheck™ low and normal controls. Two replicates of each control were analyzed in each run, and two runs were performed per day for a total of 21 days. Three different instruments with three different operators were used, each for seven of the

21 days. One reagent lot and one lot of BD FACSCount control beads were used for the duration of the study.

Coefficients of variation (CVs) and standard deviations (SDs) are provided for CD4 absolute counts and CD4 percentages for within-device* and within-run precision in Table 3 and Table 4.

Table 3 Within-device and within-run precision CD4 absolute counts

	Low control CV (cells/µL)	Normal control CV (cells/µL)
Within device	4.82	4.28
Within run	4.04	3.46

Table 4 Within-device and within-run precision CD4 percentages

	Low control SD (%)	Normal control SD (%)
Within device	0.38	1.28
Within run	0.35	1.15

Stability

A stability study was conducted at two clinical laboratories to assess the stability of the BD FACSCount CD4 reagents, and the following were measured:

- Changes associated with the storage of whole blood before staining
- Changes as a result of time between staining and data acquisition
- The combined effect of both

Whole blood samples were tested up to 24 hours post draw, and stained samples were tested up to 48 hours post stain. All samples were maintained at room temperature (20°C–25°C) before staining or acquisition.

Based on the results of this study, we recommend staining whole blood samples within 24 hours of draw and analyzing stained samples within 48 hours of staining.

Linearity

Linearity of the BD FACSCount CD4 reagent assay was assessed for the BD FACSCount instrument within a $CD4^+$ cell concentration of 50 to 5,000 cells/µL. Results were observed to be linear across the range.

Cross Reactivity

The specificity of these monoclonal antibodies has been established by blind testing at a number of laboratories by the International Leucocyte Workshop Group.[21]

User-Reportable Ranges

We conducted performance testing for the following ranges:

- Absolute counts: 50 to 5,000 $CD4^+$ cells/µL
- Percentages: 5% to 65%

Performance characteristics outside these ranges have not been established.

8. LIMITATIONS

CAUTION The pipet used in the sample preparation procedure must be properly calibrated to ensure it is dispensing exactly 50 µL of blood.

- Perform blood and control bead delivery by reverse pipetting. (The BD FACSCount pipet is preprogrammed to operate in the reverse pipetting mode.) Pipetting

* For this study, *within-device precision* has the same meaning as *total precision*.

precision and accuracy must be verified. (See the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents*).

- The vortex used must be set to a speed that causes the liquid to rise to the top of the reagent tube. Inadequate suspension of white blood cells can result in inaccurate results.
- Collect samples only in EDTA blood collection tubes. A minimum of 100 µL of whole blood is required for the test.
- Prepare samples within 24 hours of draw and analyze samples within 48 hours of preparation.
- Correct incubation time is critical and must be at least 30 minutes but no longer than 40 minutes for each sample. For this reason, we recommend preparing no more than 15 control and sample tubes at one time.
- Do not refrigerate whole blood before preparing.
- Do not dilute whole blood or use any volume other than 50 µL.
- The reagents used in this test system are light sensitive. Minimize exposing the reagent tubes to light.
- We recommend that each laboratory establish its own normal reference ranges.
- Product performance has not been established on persons undergoing monoclonal antibody chemotherapy.
- Use BD FACSCount CD4 reagents and controls only with the BD FACSCount instrument.
- Do not mix reagent lots when running controls or samples.
- BD conducted performance testing for the following ranges:
- Absolute counts: 50 to 5,000 $CD4^+$ cells/µL
- Percentage: 5% to 65%

  Performance characteristics outside these ranges have not been established. Any results outside these ranges will cause the following statement to appear on the Sample Run or Control Run printout: *Results are outside the product validated range*.

TROUBLESHOOTING

Refer to the troubleshooting section in the *BD FACSCount System User's Guide For Use with BD FACSCount CD4 Reagents* for troubleshooting information.

WARRANTY

Unless otherwise indicated in any applicable BD general conditions of sale for non-US customers, the following warranty applies to the purchase of these products.

THE PRODUCTS SOLD HEREUNDER ARE WARRANTED ONLY TO CONFORM TO THE QUANTITY AND CONTENTS STATED ON THE LABEL OR IN THE PRODUCT LABELING AT THE TIME OF DELIVERY TO THE CUSTOMER. BD DISCLAIMS HEREBY ALL OTHER WARRANTIES, EXPRESSED OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY AND FITNESS FOR ANY PARTICULAR PURPOSE AND NONINFRINGEMENT. BD'S SOLE LIABILITY IS LIMITED TO EITHER REPLACEMENT OF THE PRODUCTS OR REFUND OF THE PURCHASE PRICE. BD IS NOT LIABLE FOR PROPERTY DAMAGE OR ANY INCIDENTAL OR CONSEQUENTIAL DAMAGES, INCLUDING PERSONAL INJURY, OR ECONOMIC LOSS, CAUSED BY THE PRODUCT.

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