



BD Tritest CD3 FITC/ CD8 PE/CD45 PerCP

Reagent

50 Tests per vial—**Catalog No. 340344**
50 Tests per vial with
BD Trucount tubes—**Catalog No. 340406**

For determining percentages and absolute counts of human total T and suppressor/cytotoxic T lymphocytes in erythrocyte-lysed whole blood

For In Vitro Diagnostic Use.

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1. INTENDED USE

BD Tritest™ CD3 fluorescein isothiocyanate (FITC)/CD8 phycoerythrin (PE)/CD45 peridinin chlorophyll protein* (PerCP) is a three-color direct immunofluorescence reagent for use with a suitably equipped flow cytometer to identify and determine the percentages and absolute counts of mature human T lymphocytes (CD3+) and suppressor/cytotoxic (CD3+CD8+) T-lymphocyte subsets in erythrocyte-lysed whole blood. When used with BD Trucount™ tubes, absolute counts of these populations can be enumerated from a single tube.

This BD Tritest reagent and BD Trucount tubes can be used with the BD FACSTM Loader. The reagent can be used with or without an isotype control.

2. SUMMARY AND EXPLANATION

Human lymphocytes can be divided into three major populations based on their biologic function and cell-surface antigen expression: T lymphocytes, B lymphocytes, and natural killer (NK) lymphocytes.

Clinical Applications

Suppressor/cytotoxic lymphocytes are a subset of T lymphocytes (CD3+) that are CD8+. CD3+CD8+ percentages or counts are used to characterize and monitor some forms of immunodeficiency¹⁻³ and autoimmune diseases.^{4,5}

The percentage of suppressor/cytotoxic lymphocytes lies outside the normal reference range in some autoimmune diseases.⁶ The relative percentage of the CD8+ subset is elevated in many patients with congenital or acquired immune deficiencies such as severe combined immunodeficiency

* Patents—PerCP: US 4,876,190

(SCID)¹ or acquired immunodeficiency syndrome (AIDS).⁷

The Centers for Disease Control (CDC) recommends using reagent combinations containing CD3 and CD8 antibodies for determining the percentage of CD8⁺ T lymphocytes in human immunodeficiency virus (HIV)-infected patients.⁸

3. PRINCIPLES OF THE PROCEDURE

When whole blood is added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leucocyte surface antigens. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. BD Tritest reagents use fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population⁹⁻¹¹ to reduce contamination of unlysed or nucleated red blood cells in the gate.

When BD Trucount tubes are used, a precise volume of sample is stained directly in a BD Trucount tube. The lyophilized pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/ μ L) of positive cells in the sample can be determined by comparing cellular events to bead events. If appropriate software such as BD Multiset™ software is used, absolute counts will be determined by the software. If manually performing data analysis using software such as BD CellQuest™ software, simply divide the number of positive cellular events by the number of bead events, then multiply by the BD Trucount bead concentration.

4. REAGENT

Reagent Provided, Sufficient for 50 Tests

BD Tritest CD3/CD8/CD45 reagent is provided in 1 mL of buffered saline with bovine serum albumin and 0.1% sodium azide. It contains FITC-labeled CD3, clone SK7;¹²⁻¹⁴ PE-labeled CD8, clone SK1;^{15,16} and PerCP-labeled CD45, clone 2D1 (HLe-1).¹⁷

CD3 identifies T lymphocytes and recognizes the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex.¹⁸ This complex is composed of at least six proteins that range in molecular weight from 20 to 30 kilodaltons (kd).¹⁹ The antigen recognized by CD3 antibodies is noncovalently associated with either α/β or γ/δ TCR (70 to 90 kd).²⁰

CD8 identifies suppressor/cytotoxic T lymphocytes and recognizes an antigen expressed on the 32-kd α subunit of a disulfide-linked bimolecular complex.²¹ The cytoplasmic domain of the α subunit of the CD8 antigen is associated with the protein tyrosine kinase p56^{lck}.²² The CD8 molecule interacts with class I major histocompatibility complex (MHC) molecules resulting in increased adhesion between the CD8⁺ T lymphocytes and the target cells.²³⁻²⁵ Binding of the CD8 molecule to class I MHC molecules enhances the activation of resting T lymphocytes.²³⁻²⁵

CD45 identifies leucocytes and recognizes a 180- to 220-kd human leucocyte antigen that is a member of the leucocyte common antigen (LCA) family.²⁶

CD3, CD8, and CD45 antibodies are composed of mouse γ_1 heavy chains and kappa light chains.

BD Trucount tubes contain a freeze-dried pellet of fluorescent beads in a single-use

tube. Each BD Trucount pouch contains 25 tubes, sufficient for 25 tests.

Precautions

- For In Vitro Diagnostic Use.
- Do not use the reagent if you observe any change in appearance. Precipitation or discoloration indicates instability or deterioration.
- The antibody reagent contains sodium azide as a preservative; however, take care to avoid microbial contamination, which can cause erroneous results.

WARNING Sodium azide is harmful if swallowed (R22). Keep away from food, drink, and animal feedstuff (S13). Wear suitable protective clothing (S36). If swallowed, seek medical advice immediately and show this container or label (S46). Contact with acids liberates very toxic gas (R32). Azide compounds should be flushed with large volumes of water during disposal to avoid deposits in lead or copper plumbing where explosive conditions can develop.

- **WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection^{27,28} and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing and gloves. Fixation has been reported to inactivate HIV.²⁹
- BD FACSTM lysing solution* is required and contains diethylene glycol and formaldehyde. Refer to the package insert for warnings.

- If BD Trucount tubes are used, the addition of a precise volume of blood is critical to achieving an accurate result. Calibrate pipettes to deliver exactly 50 μ L of sample, or perform the reverse pipetting technique (see step 3 on page 5 for a brief description). Refer to the pipette manufacturer's instructions for more information.
- Bead count varies by lot of BD Trucount tubes. It is critical to use the bead count shown on the current lot of BD Trucount tubes when entering this value in the software or when manually calculating absolute counts. Do not mix multiple lots of tubes in the same assay.
- BD Trucount tubes are designed for use with a specific lyse/no-wash procedure. Do not attempt to threshold on forward scatter (FSC) for data collection.

Storage and Handling

- Store the reagent at 2° to 8°C. Do not use after the expiration date shown on the label.
- Do not freeze the reagent or expose it to direct light during storage or incubation with cells. Keep the reagent vial dry.
- Store BD Trucount tubes in their original foil pouch at 2° to 25°C. To avoid potential condensation, open the pouch only after it has reached room temperature and carefully reseal the pouch immediately after removing a tube. Examine the desiccant each time you open the pouch. If the desiccant has turned from blue to lavender, discard the remaining tubes. An unopened pouch is stable until the expiration date shown on the

* US Patent Nos. 4,654,312; 4,902,613; 5,098,849

packaging. Do not open and use tubes after this expiration date. Use tubes within 1 hour after removal from the foil pouch. Use remaining tubes within 1 month after opening the pouch.

5. INSTRUMENT

BD Tritest CD3/CD8/CD45 reagent and BD Trucount tubes are designed for use on a flow cytometer equipped with appropriate computer hardware and software. BD recommends the BD FACSCalibur™, BD FACSort™, or BD FACScan™ flow cytometers; however, results can be achieved using other platforms. The flow cytometer must be equipped with a 488-nm laser capable of detecting light scatter (forward and side) and three-color fluorescence with emission detectable in three ranges: 515–545 nm, 562–607 nm, and >650 nm. The instrument must be able to threshold or discriminate using the >650-nm channel. The BD FACS Loader can also be used with this product.

Use BD Calibrite™ beads and BD FACSComp™ software, version 2.0 or later, for setting the photomultiplier tube (PMT) voltages, setting the fluorescence compensation, and checking instrument sensitivity before use. For users of flow cytometers manufactured by companies other than BD, refer to the manufacturer's instructions for setting up three-color immunophenotyping.

BD has developed software applications, such as BD Multiset software, that automatically calculate absolute counts when BD Trucount tubes are used. However, other software packages can be used for data acquisition and analysis and the absolute counts can be calculated manually.

6. SPECIMEN COLLECTION AND PREPARATION

Collect blood aseptically by venipuncture^{30,31} into a sterile EDTA (ethylenediamine-tetraacetic acid) BD Vacutainer™ blood collection tube. BD Tritest CD3/CD8/CD45 reagent and BD Trucount tubes have been validated with both liquid and dry formulations of EDTA.

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 BD Trucount beads.

Obtain a white blood cell (WBC) count and a differential white cell count from the same whole blood sample before staining to ensure that the WBC count is within the linear range (see Linearity on page 9) or to calculate absolute counts from percentages.

Anticoagulated blood stored at room temperature (20° to 25°C) must be stained within 48 hours of draw and then analyzed within 6 hours of staining. If samples are stained within 24 hours of draw, they can be analyzed within 24 hours of staining.

Interfering Conditions

Do not use previously fixed and stored patient specimens. Whole blood samples refrigerated before staining can give aberrant results. Samples obtained from patients taking immunosuppressive drugs can yield poor resolution.³² Blast cells can interfere with test results. Hemolyzed samples should be rejected.

7. PROCEDURE

Reagent Provided

- BD Tritest CD3 FITC/CD8 PE/CD45 PerCP (BD Catalog No. 340344), or
- BD Tritest CD3 FITC/CD8 PE/CD45 PerCP with BD Trucount tubes (BD Catalog No. 340406)

Reagents and Materials Required but Not Provided

- BD Calibrite 3 beads (BD Catalog No. 340486)
- BD FACS lysing solution (10X), 100 mL (BD Catalog No. 349202)

Refer to the package insert for dilution instructions and warnings.

- Reagent-grade (distilled or deionized) water
- BD Vacutainer EDTA blood collection tubes
- Disposable 12 x 75-mm BD Falcon™ capped polystyrene test tubes (BD Catalog No. 352058), or equivalent if not using BD Trucount tubes
- Vortex mixer
- Micropipettor with tips
- Bulk dispenser or pipettor (450 µL) for dispensing BD FACS lysing solution
- Sheath fluid (BD FACFlow™, BD Catalog No. 342003, or equivalent)
- BD Trucount controls (BD Catalog No. 340335), necessary if using BD Trucount tubes
- Lysable whole blood control (available commercially)

Staining the Cells

BD Tritest reagents can be used with or without an isotype control to assess the

amount of nonspecific antibody binding. If you want to use a control, BD Tritest γ_1/γ_1 /CD45 isotype control reagent (BD Catalog No. 340385) is available.

Staining

1. For each patient sample, label a 12 x 75-mm tube with the sample identification number.

For absolute counts, label a BD Trucount tube in place of the 12 x 75-mm tube.

NOTE Before use, verify that the BD Trucount bead pellet is intact and within the metal retainer at the bottom of the tube. If this is not the case, discard the BD Trucount tube and replace it with another. Do not transfer beads to another tube.

2. Pipette 20 µL of BD Tritest CD3/CD8/CD45 reagent into the bottom of the tube.

If using a BD Trucount tube, pipette just above the stainless steel retainer. Do not touch the pellet.

3. Pipette 50 µL of well-mixed, anticoagulated whole blood into the bottom of the tube.

NOTE Avoid smearing blood down the side of the tube. If whole blood remains on the side of the tube, it will not be stained with the reagent and can affect results.

Accurate pipetting is critical when using a BD Trucount tube. Use the reverse pipetting technique to pipette sample onto the side of the tube just above the retainer.

For reverse pipetting, depress the button to the second stop. When you release the button, excess sample is

drawn up into the tip. Press the button to the first stop to expel a precise volume of sample; this leaves excess sample in the tip.

4. Cap the tube and vortex gently to mix. Incubate for 15 minutes in the dark at room temperature (20° to 25°C).
5. Add 450 μ L 1X BD FACS lysing solution to the tube.
6. Cap the tube and vortex gently to mix.
7. Incubate for 15 minutes in the dark at room temperature (20° to 25°C).

The sample is now ready to be analyzed on the flow cytometer.

Flow Cytometry

If samples are not to be analyzed immediately after preparation, store them in the dark at room temperature (20° to 25°C).

Vortex the cells thoroughly (at low speed) to reduce aggregation before running them on the flow cytometer.³³ If you are using the BD FACS Loader, vortex tubes immediately before placing them into the loader racks. Acquire and analyze data using the appropriate software, such as BD CellQuest or BD Multiset software. Before acquiring samples, adjust the threshold to minimize debris and ensure populations of interest are included.

Quality Control

Run a control sample daily from a normal adult subject or a commercially available whole blood control to optimize instrument settings and as a quality control check of the system.³¹ The BD Tritest isotype control reagent is optional to set fluorescence markers for detecting the presence of nonspecific staining.

Use commercial controls with established values for percent positive and absolute counts with each run to assess system performance.

Visually inspect the CD45 vs SSC dot plot. The lymphocyte population should appear as a bright, compact cluster with low SSC. Monocytes and granulocytes should also appear as distinct clusters. Do not proceed with analysis if populations are diffuse and there is little or no separation between clusters.

See Figure 1 and Figure 2 and Figure 3 (page 7) for representative data from a hematologically normal adult sample stained with BD Tritest CD3/CD8/CD45 in a BD Trucount tube.

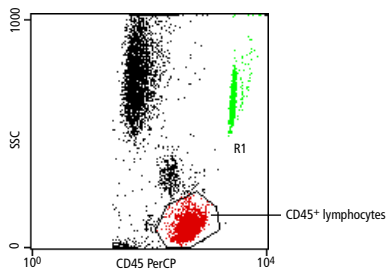


Figure 1 Ungated CD45 vs SSC dot plot

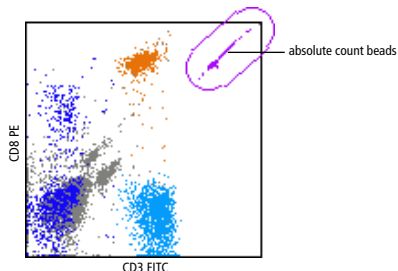


Figure 2 Ungated CD3 vs CD8 dot plot

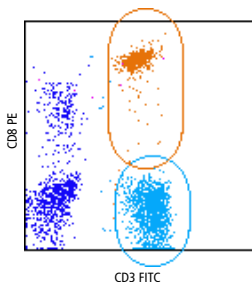


Figure 3 Lymphocyte-gated CD3 vs CD8 dot plot

8. RESULTS

Results are reported as the percentage of positive cells per lymphocyte population or as the number of positive cells per microliter of blood (absolute count).

Calculating Absolute Counts

During analysis, the absolute number (cells/ μ L) of positive cells in the sample can be determined by comparing cellular events to bead events. If BD Multiset software is used, absolute counts will be determined automatically.

For manual data analysis using BD CellQuest or other software, simply divide the number of positive cellular events by the number of bead events, and then multiply by the BD Trucount bead concentration.

$$\frac{\text{\#events in cell population}}{\text{\#events in absolute count bead region}} \times \frac{\text{\#beads/test}^*}{\text{test volume}} = \text{cell population absolute count}$$

* This value is found on the BD Trucount tube foil pouch label and can vary from lot to lot.

9. LIMITATIONS

- Laboratories must establish their own normal reference ranges for the

BD Tritest CD3/CD8/CD45 reagent parameters that can be affected by gender of patient, age of patient, and preparative technique. Race of patient³⁴ and individual variations of epitope expression³⁵ can also have an effect, although sufficient data is not available to establish this. Age, gender, clinical characteristics, and race of patients should be known when a reference range is determined.³⁶ Reference ranges provided are for information only.

- BD Tritest CD3/CD8/CD45 reagent has not been validated by BD Biosciences for use with heparin or acid citrate dextrose (ACD) liquid anticoagulants in determining absolute counts with BD Trucount tubes.
- BD Tritest CD3/CD8/CD45 reagent is not intended for screening samples for the presence of leukemic cells or for use in phenotyping samples from leukemia patients.
- Absolute counts are not comparable between laboratories using different manufacturer's equipment.

10. EXPECTED VALUES

Reference Ranges

The reference ranges for CD3/CD8/CD45 shown in Table 1 were determined at BD Biosciences in San Jose, CA, and at four clinical centers: Cleveland Clinic Foundation, Cleveland, OH; Johns Hopkins Hospital, Baltimore, MD; Institute of Tropical Medicine, Antwerp, Belgium; and University of North Carolina Hospital, Chapel Hill, NC. Subjects were hematologically normal adults between the ages of 18 and 65 years.

These reference ranges are pooled ranges. For detailed information about reference ranges obtained with this study, contact BD Customer Support. Refer to the first limitation for more information about reference ranges.

11. PERFORMANCE CHARACTERISTICS

Performance of the reagents was established by testing at BD Biosciences laboratories in San Jose, CA, at an external clinical center in the US or Europe, or at a combination of sites.

Table 1. Representative reference ranges, BD Tritest CD3/CD8/CD45, in hematologically normal adults

Subset	n	Mean	Lower 2.5 Percentile	Upper 97.5 Percentile
Suppressor/cytotoxic T lymphs (%)	523	25	13	41
Total T lymphs (%)	516	72	55	84
Suppressor/cytotoxic T lymphs (cells/ μ L) ^a	523	490	190	1,140
Total T lymphs (cells/ μ L) ^a	516	1,410	690	2,540

a. Absolute counts rounded to the nearest 10 cells/ μ L

Accuracy

Lymphocyte subset percentage enumerations with BD Tritest CD3/CD8/CD45 were compared with results from BD Simultest™ CD3/CD8. Absolute counts were compared to results from the BD FACSCount™ instrument.

Aliquots of the same blood sample from normal and abnormal donors were analyzed. Regression statistics reported in Table 2 indicate that the results are substantially equivalent.

Table 2. Regression analysis

Subset	n	Slope	Intercept	r	Range
Suppressor/cytotoxic T lymphs (%)	168	0.98	0.2%–positive	0.99	15–84
Total T lymphs (%)	168	0.92	6.3%–positive	0.97	26–94
Suppressor/cytotoxic T lymphs (cells/ μ L)	194	1.06	–10 cells/ μ L	0.98	70–1,980 ^a
Total T lymphs (cells/ μ L)	197	1.04	–11 cells/ μ L	0.99	120–2,860 ^a

a. Absolute counts rounded to the nearest 10 cells/ μ L

Within-Specimen Reproducibility

Ten aliquots of specimens from three samples representing high, medium, and low CD4 counts were assessed. The %-positive results were as follows (SD = standard deviation):

- %CD3: mean = 71, pooled SD = 1.1
- %CD8: mean = 22, pooled SD = 1.0

The results for absolute counts are shown in Table 3.

Table 3. Within-specimen reproducibility for BD Tritest CD3/CD8/CD45 reagent

Subset	Level	Mean	SD	%CV
Suppressor/cytotoxic T lymphs (cells/ μ L)	High	713	82.2	11.5
	Med	455	27.5	6.1
	Low	321	29.9	9.3
Total T lymphs (cells/ μ L)	High	2,734	262.7	9.6
	Med	1,851	84.9	4.6
	Low	708	51.2	7.2

Stability

A stability study was conducted to assess the effect of time with respect to BD Tritest reagent performance specifications. The study measured: 1) changes associated with the storage of whole blood before staining, 2) changes as a result of time between staining and data acquisition, and 3) the combined effect of the two.

Based on the results of this study,* we recommend staining samples within 48 hours of draw and analyzing samples within 6 hours of staining; or staining samples within 24 hours of draw and analyzing within 24 hours of staining.

Cross-Reactivity

The CD8 antibody reacts with NK lymphocytes³⁷ as well as with suppressor/cytotoxic T lymphocytes.

Linearity

The linearity was assessed by testing within a WBC concentration of 2.5×10^3 to 31×10^3 WBC/ μ L and a lymphocyte concentration of 2.0×10^2 to 16.7×10^3 lymphocytes/ μ L. Results were observed to be linear within the CD3+CD8+ range (43 to 3.9×10^3 cells/ μ L) and the CD3+ range (122 to 11.2×10^3 cells/ μ L).

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.

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* Data available at BD Biosciences

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