BD Trucount™ Tubes

For determining absolute counts of leucocytes in blood

Catalog No. 340334

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IVD

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1. INTENDED USE

BD Trucount™ Tubes are used for determining absolute counts of leucocytes in blood.

BD Trucount™ Tubes are designed for use with in vitro diagnostic products such as BD Tritest™ reagents, and a suitably equipped flow cytometer. BD Trucount™ Tubes can be used with the BD FACS™ Loader.

2. PRINCIPLES OF THE PROCEDURE

Procedures described in this instructions for use (IFU) apply to immunophenotyping applications. For other applications, refer to the appropriate product-specific IFU.

Add the appropriate monoclonal antibody reagent and whole blood directly to the BD Trucount™ Tube. The lyophilized pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/µL) of positive cells in the sample can be determined by comparing cellular events to bead events. If the appropriate software, such as BD Multiset™, is used, absolute counts will be determined by the software. If you are manually performing data analysis using software such as BD CellQuest™ Pro, simply divide the number of positive cellular events by the number of bead events, then multiply by the BD Trucount™ bead concentration.

3. REAGENT

Each box contains two pouches. Each pouch contains 25 BD Trucount™ Tubes, sufficient for 25 tests.

Precautions

- For In Vitro Diagnostic Use.
- BD Trucount™ Tubes are designed for use with a specific lyse/no-wash procedure. For absolute counting, prepare and analyze samples within BD Trucount™ Tubes. Do not transfer beads to another tube. The presence of proteins, such as serum proteins contained in whole blood, is necessary for proper performance of BD Trucount™ beads in absolute counting.[1] Follow specific assay IFUs when diluting samples. **Do not attempt to threshold on forward scatter (FSC) for data collection.** Do not remove the metal retainer in the BD Trucount™ Tube.
- It is the responsibility of the user to validate any other method or use.
- The addition of a precise volume of blood is critical to achieving the result. Pipettes must be calibrated to deliver exactly 50 µL of sample. If this or a similar type of pipette is not used, perform the reverse pipetting technique (see Reverse Pipetting in Section 6 for a brief description). Refer to the pipette manufacturer's instructions for more information.
- Always be sure to use the bead count from the current lot of BD Trucount™ Tubes when entering this value in the software or when manually calculating an absolute count. The correct bead count is critical for determining a cell count. Do not mix multiple lots of tubes in the same assay.
- Store BD Trucount™ Tubes in their original foil pouch at 2°C–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. An unopened pouch is stable until the expiration date shown on the packaging. Do not open the pouch and use tubes after the expiration date. Use tubes within 1 hour after removal from the foil pouch. Use remaining tubes within 1 month after opening the pouch.

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection[2,3] 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.

BD FACS™ Lysing Solution is required and contains diethylene glycol and formaldehyde. Refer to the *BD FACS™ Lysing Solution* IFU for warnings.

4. INSTRUMENTS

BD Trucount™ applications are designed for flow cytometers equipped with appropriate computer hardware and software. The flow cytometer must be equipped to detect three-color fluorescence, forward scatter (FSC), and side scatter (SSC). We recommend the BD FACSCalibur™ flow cytometer; however, results can be achieved using other platforms. Refer to the appropriate reagent IFU for specific instrument limitations. The BD FACS™ Loader can also be used with this product. BD has developed BD Multiset™ software, for use with specific reagents and BD Trucount™ Tubes, which automatically calculates absolute counts. However, you can also use software such

as BD CellQuest™ Pro for data acquisition and analysis and manually calculate absolute counts.

5. SPECIMEN COLLECTION AND PREPARATION

Collect blood aseptically by venipuncture[4,5] into a sterile EDTA (lavender top) BD Vacutainer® blood collection tube. Follow the collection tube manufacturer's guidelines for the minimum volume of blood to be collected. Store anticoagulated blood at room temperature (20°C– 25°C) until ready for staining.

6. PROCEDURE

Reagent Provided

BD Trucount™ Tubes (Catalog No. 340334), containing a freeze-dried pellet of fluorescent beads in a single-use tube.

Reagents and Materials Required but Not Provided

- BD FACSComp™ Beads

  Refer to your product catalog for information on the specific BD FACSComp™ product for your application.

- BD FACS™ Lysing Solution (10X), 100 mL (Catalog No. 349202)

  Refer to the *BD FACS™ Lysing Solution* IFU for dilution instructions and warnings.

- Reagent-grade (distilled or deionized) water
- EDTA BD Vacutainer® blood collection tubes, or equivalent
- Vortex mixer
- Micropipettor with tips
- Bulk dispenser or pipettor (450 µL) for dispensing BD FACS™ Lysing Solution
- BD FACSFlow™ Sheath Fluid (Catalog No. 342003), or equivalent

Staining the Cells

Stain whole blood samples following specific instructions in the appropriate reagent IFU. Lyse red blood cells after staining using diluted (1X) BD FACS™ Lysing Solution. Use care to protect the tubes from direct light. Perform the procedure at room temperature (20°C–25°C).

Reverse Pipetting

A precise volume of whole blood is critical. If a pipette that delivers a precise volume of blood is not used, perform reverse pipetting. This technique takes advantage of two stops in a pipette.

- For normal pipetting, typically, the button is depressed to the first stop; sample is drawn up by releasing the button, then expelled by pressing to the first stop again.
- For reverse pipetting, the button is depressed to the second stop. When the button is released, excess sample is drawn up into the tip. A precise volume of sample is expelled by pressing the button to the first stop, leaving excess sample in the tip.

Staining

Refer to the appropriate reagent IFU for detailed sample preparation instructions.

1. For each patient sample, label a BD Trucount™ Tube with the reagent and sample identification number.

   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.

2. Pipette 20 µL of the appropriate reagent just above the stainless steel retainer. Do not touch the pellet.
3. Pipette 50 µL of well-mixed, anticoagulated whole blood onto the side of the tube just above the retainer.

   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.

   Accuracy is critical. Use a BD electronic pipette or use the reverse pipetting technique to pipette sample onto the side of the tube just above the retainer.
4. Cap the tube and vortex gently to mix. Incubate for 15 minutes in the dark at room temperature (20°C–25°C).
5. Add 450 µL 1X BD FACS™ Lysing Solution to the tube.
6. Cap the tube and vortex gently to mix. Incubate for 15 minutes in the dark at room temperature. The sample is now ready to be analyzed on the flow cytometer.

Flow Cytometry

Refer to the appropriate reagent IFU for specific instructions. Vortex the samples thoroughly (at low speed) to resuspend beads and reduce cell aggregation before running them on the flow cytometer.[6] If using the BD FACS™ Loader for acquisition, vortex tubes immediately before placing them into the Loader racks. Acquire and analyze list-mode data using the appropriate software.

We recommend using Calibrite beads and the appropriate software such as BD FACSComp™, version 2.0 or later, for setting the photomultiplier tube (PMT) voltages, setting the fluorescence compensation, and checking instrument sensitivity prior to use.

Before acquiring samples, adjust the threshold to minimize debris and ensure populations of interest are included. Figures 1, 2, and 3 show an example of BD Trucount™ Tubes used with BD Tritest™ CD3/CD4/CD45 reagent.

If you are not using a BD software program that automatically calculates absolute cell counts, you can obtain the absolute count of the cell population (A), by dividing the number of positive cell events (X) by the number of bead events (Y), and then multiplying by the BD Trucount™ bead concentration (N/V, where N = number of beads per test* and V = test volume). $A = X/Y \times N/V$

Gate the lymphocyte population (2) from an FL3 vs SSC dot plot. Then, obtain the number of events in the quadrant or region containing the cell population from a gated FL1 vs FL2 dot plot (see Figure 2). Obtain the number of events in the absolute count bead (1) region from an ungated FL1 vs FL2 dot plot (see Figure 3).

* This value is found on the BD Trucount™ Tubes foil pouch label and might vary from lot to lot.

Figure 1 FL3 (CD45) vs SSC dot plot

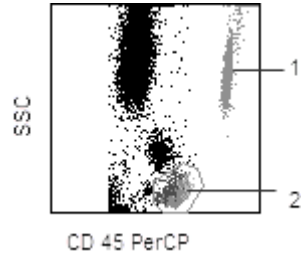


Figure 2 Gated FL1 vs FL2 dot plot

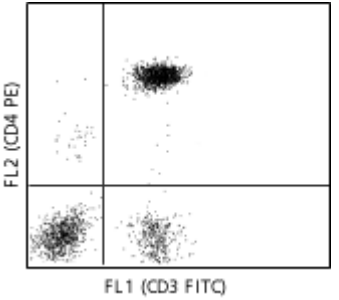
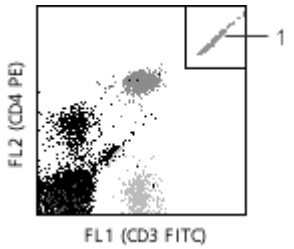


Figure 3 Ungated FL1 vs FL2 dot plot



Quality Control

Run a control sample daily from a normal adult subject to optimize instrument settings and as a quality control check of the system.[5]

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.

7. PERFORMANCE CHARACTERISTICS

Performance was established by comparison with the BD FACSCount™ system. These results with BD Tritest™ CD3/CD4/CD45 are representative of results obtained with other IVD phenotyping reagents. Refer to specific reagent IFUs for more details.

Accuracy

Whole blood was stained with BD Tritest™ CD3/CD4/CD45 using BD Trucount™ Tubes and acquired and analyzed using BD CellQuest™ Pro software. Two samples of each specimen were stained and analyzed in parallel using the BD FACSCount™ system. Data was analyzed to determine the average differences between the results obtained using BD Tritest™/BD Trucount™ versus results with BD FACSCount™. The results appear in Figure 4 and Figure 5, and Table 1 and Table 2.

Figure 4 BD FACSCount™ results versus BD Tritest™/BD Trucount™ results

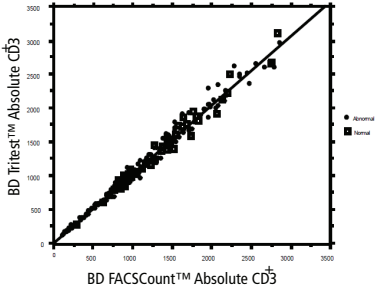


Figure 5 BD FACSCount™ results versus BD Tritest™/BD Trucount™ results

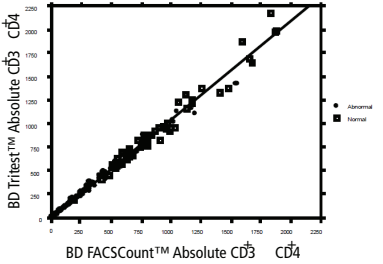


Table 1 Regression Analysis: BD Tritest™ $CD3^+$ (cells/µL) vs BD FACSCount™ $CD3^+$

Parameter	Parameter Estimate	90% Lower Confidence Limit	90% Upper Confidence Limit
Y-intercept	–7.0	–28	14
Slope	1.03	1.01	1.04

r = 0.99; n = 197

Table 2 Regression analysis: BD Tritest™ $CD3^+CD4^+$ (cells/µL) vs BD FACSCount™ $CD3^+CD4^+$

Parameter	Parameter Estimate	90% Lower Confidence Limit	90% Upper Confidence Limit
Y-intercept	1.2	-6.7	9.1
Slope	1.04	1.03	1.06

r = 0.99; n = 199

Precision

A study using ten replicates each of a low, medium, and high sample was performed to assess reproducibility. The results appear in Table 3.

Table 3 Reproducibility: BD Trucount™ with BD Tritest™ CD3/CD4/CD45

Sample	n	Subset	Mean	CV%[a]
Low	10	$CD3^+$	704	7.0
		$CD3^+CD4^+$	371	7.1
Medium	10	$CD3^+$	1,897	4.1
		$CD3^+CD4^+$	1,352	3.9
High	10	$CD3^+$	2,716	4.4
		$CD3^+CD4^+$	2,034	4.2

a. CV = coefficient of variation

Precision studies were also performed at three external clinical sites to assess absolute count within-sample reproducibility for normal and abnormal samples. For each sample, three aliquots of whole blood were stained with BD Tritest™ reagents using BD Trucount™ Tubes. Examples of

randomly selected results for individual subjects appear in Table 4.

Table 4 CD3/CD4/CD45 with BD Trucount™ representative samples (cells/µL, n = 3)

Table 4a: Site 1						
	Mean CD4	SD[a]	CV%[b]	Mean CD3	SD[a]	CV%[b]
Low	10.0	3.8	38.4	287	3.9	1.4
	209	11.1	5.3	1,206	21.3	1.8
	427	32.2	7.5	3,051	181.2	5.9
Med	502	34.6	6.9	1,784	97.5	5.5
	701	16.6	2.4	926	15.5	1.7
	961	102.1	10.6	3,319	252.9	7.6
High	1,129	42.7	3.8	2,184	71.3	3.3
	1,142	45.1	4.0	1,925	80.8	4.2

a. SD = standard deviation
b. CV = coefficient of variation

Table 4b: Site 2						
	Mean CD4	SD[a]	CV%[b]	Mean CD3	SD[a]	CV%[b]
Low	86	7.6	8.9	768	29.5	3.8
	266	24.6	9.2	1,047	53.5	5.1
	422	23.3	5.5	3,533	247.6	7.0
Med	517	12.3	2.4	1,083	7.0	0.6
	689	27.7	4.0	1,623	126.5	7.8
	903	45.4	5.0	1,589	67.6	4.3
High	1,197	93.4	7.8	2,254	149.8	6.6
	1,201	37.4	3.1	1,758	40.5	2.3
	1,363	66.7	4.9	2,330	115.5	5.0

a. SD = standard deviation
b. CV = coefficient of variation

Table 4c: Site 3						
	Mean CD4	SD[a]	CV%[b]	Mean CD3	SD[a]	CV%[b]
Low	10	1.0	10.4	267	10.5	4.0
	49	3.2	6.4	1,385	80.2	5.8
	236	11.7	5.0	1,748	46.6	2.7

Table 4c: Site 3						
	Mean CD4	SD[a]	CV%[b]	Mean CD3	SD[a]	CV%[b]
Med	715	16.8	2.3	2,214	64.2	2.9
	878	40.3	4.6	1,328	49.9	3.8
	947	61.1	6.4	1,590	120.1	7.6
High	1,056	43.5	4.1	2,904	164.5	5.7
	1,299	79.0	6.1	1,970	121.8	6.2
	1,502	65.3	4.3	2,303	63.6	2.8

a. SD = standard deviation
b. CV = coefficient of variation

At these clinical sites, the range of CVs for $CD3^+$ cells, observed for all samples, was <1% (count of 1,083 cells/µL) to 13% (count of 187 cells/µL). The range of CVs for $CD3^+CD4^+$ cells, observed for all samples, was <1% (count of 271 cells/µL) to 80% (count of 24 cells/µL).

Refer to the appropriate reagent IFU for more information about specific reagent performance.

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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REFERENCES

1. Brando B, Göhde W Jr Scarpati B, D'Avanzo G. The "vanishing counting bead" phenomenon: effect on absolute CD34+ cell counting in phosphate-buffered saline–diluted leukapheresis samples. *Cytometry*. 2001;43:154-160.
2. *Protection of Laboratory Workers from Occupationally Acquired Infections; Approved*

Guideline — Fourth Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2014. CLSI document M29-A4.

3. Centers for Disease Control and Prevention. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. https://www.cdc.gov/infectioncontrol/guidelines/isolation/index.html. Accessed March 12, 2019.
4. *Collection of Diagnostic Venous Blood Specimens; Approved Standard -- Seventh Edition*. Wayne, PA: Clinical and Laboratory Standards Institute; 2017. CLSI document GP41-Ed7.
5. Enumeration of Immunologically Defined Cell Populations by Flow Cytometry; Approved Guideline–Second Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2007. CLSI document H42-A2.
6. Jackson AL, Warner NL. Preparation, staining, and analysis by flow cytometry of peripheral blood leukocytes. In: Rose NR, Friedman H, Fahey JL, eds. *Manual of Clinical Laboratory Immunology*. 3rd ed. Washington, DC: American Society for Microbiology; 1986:226-235.