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 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. 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. Examine the desiccant each time you open the pouch. If the desiccant has turned from blue to lavender, discard the remaining tubes. Use tubes within 1 hour after removal from the foil pouch and do not use beyond the expiration date indicated on the packaging.

**WARNING** All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection 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™ lysis solution is required and contains diethylene glycol and formaldehyde. Refer to the BD FACS Lysing Solution instructions for use for warnings.

### 4. INSTRUMENT

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 (Catalog No. 356457), 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.
- BD Trucount™ Controls (Catalog No. 340335).

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. 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 BD FACSComp™ 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). 

\[ A = \frac{X}{Y} \times \frac{N}{V} \]

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).

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* This value is found on the BD Trucount tube foil pouch label and might vary from lot to lot.
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.\textsuperscript{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\textsuperscript{TM} 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+

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Estimate</th>
<th>90% Lower Confidence Limit</th>
<th>90% Upper Confidence Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-intercept</td>
<td>-7.0</td>
<td>-28</td>
<td>14</td>
</tr>
<tr>
<td>Slope</td>
<td>1.03</td>
<td>1.01</td>
<td>1.04</td>
</tr>
</tbody>
</table>

r = 0.99; n = 197

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter Estimate</th>
<th>90% Lower Confidence Limit</th>
<th>90% Upper Confidence Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y-intercept</td>
<td>1.2</td>
<td>-6.7</td>
<td>9.1</td>
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<tr>
<td>Slope</td>
<td>1.04</td>
<td>1.03</td>
<td>1.06</td>
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</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>n</th>
<th>Subset</th>
<th>Mean</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>10</td>
<td>CD3+</td>
<td>704</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD3+CD4+</td>
<td>371</td>
<td>7.1</td>
</tr>
<tr>
<td>Medium</td>
<td>10</td>
<td>CD3+</td>
<td>1,897</td>
<td>4.1</td>
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<tr>
<td></td>
<td></td>
<td>CD3+CD4+</td>
<td>1,352</td>
<td>3.9</td>
</tr>
<tr>
<td>High</td>
<td>10</td>
<td>CD3+</td>
<td>2,716</td>
<td>4.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CD3+CD4+</td>
<td>2,034</td>
<td>4.2</td>
</tr>
</tbody>
</table>

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>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>CD4</td>
<td>3.8</td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td>209</td>
<td>3.8</td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td>427</td>
<td>3.8</td>
<td>38.4</td>
</tr>
<tr>
<td>Med</td>
<td>CD4</td>
<td>3.8</td>
<td>38.4</td>
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<tr>
<td></td>
<td>701</td>
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</tr>
<tr>
<td></td>
<td>961</td>
<td>3.8</td>
<td>38.4</td>
</tr>
<tr>
<td>High</td>
<td>CD4</td>
<td>3.8</td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td>1,129</td>
<td>3.8</td>
<td>38.4</td>
</tr>
<tr>
<td></td>
<td>1,142</td>
<td>3.8</td>
<td>38.4</td>
</tr>
</tbody>
</table>

a. SD = standard deviation
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.