1. INTENDED USE
BD Trucount™ tubes are intended for use with appropriate BD IVD reagent products on BD FACSLyric™, BD FACSVia™, BD FACSCanto™ II, BD FACSCanto™, and BD FACS_Calibur™ flow cytometers to determine the absolute counts of leucocytes in erythrocyte-lysed whole blood.

2. SUMMARY AND EXPLANATION
BD Trucount tubes contain a known number of fluorescent beads, which is specified on the pouch label. The beads are used to determine absolute counts of leucocytes in blood.

3. PRINCIPLES OF THE PROCEDURE
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.

4. REAGENT
Each pouch contains 25 BD Trucount tubes, sufficient for 25 tests.
Precautions

- 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 instructions for use (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.

- When using BD Trucount tubes, the addition of a precise volume of sample is critical. Pipets must be calibrated to deliver exactly 50 µL of sample. We recommend performing the reverse pipetting technique according to the pipet manufacturer’s instructions.

- 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.

5. STORAGE AND HANDLING

Store BD Trucount tubes in their original foil pouch at 2ºC–25ºC. 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. Once the pouch has been opened, the tubes are stable for 1 month. Do not use beyond the expiration date indicated on the packaging.

6. 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, FSC, and side scatter (SSC).

Table 1 Recommended BD systems

<table>
<thead>
<tr>
<th>Flow cytometer</th>
<th>Setup software</th>
<th>Analysis software</th>
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</thead>
<tbody>
<tr>
<td>BD FACSlyte</td>
<td>BD FCSuite™ clinical software</td>
<td>BD FCSuite Clinical software</td>
</tr>
<tr>
<td>BD FACSVia</td>
<td>BD FACSVia™ clinical software</td>
<td>BD FACSVia clinical software</td>
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<tr>
<td>BD FACS Canto</td>
<td>BD FACS Canto™ clinical software</td>
<td>BD FACS Canto clinical software</td>
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<tr>
<td>BD FACS Canto II</td>
<td>BD FACS Canto™ clinical software</td>
<td>BD FACS Canto clinical software</td>
</tr>
<tr>
<td>BD FACS Calibur</td>
<td>BD FACSComp™ software v4.0 or later</td>
<td>BD MultiSet™ software</td>
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<tr>
<td></td>
<td></td>
<td>BD CellQuest Pro</td>
</tr>
</tbody>
</table>
7. REAGENTS AND MATERIALS

Reagent Provided
BD Trucount tubes, containing a freeze-dried pellet of fluorescent beads in a single-use tube.

Reagents and Materials Required but Not Provided
- Reagent of choice, for example, the BD Multitest™ IMK kit (Catalog No. 662965)
  See the reagent IFU for more information.
- Vortex mixer
- Micropipettor with tips

8. INSTRUCTIONS FOR USE

Performing Reverse Pipetting
Accurate pipetting is critical when using a BD Trucount tube. We recommend using the reverse pipetting technique to add the sample to a BD Trucount tube. For reverse pipetting, depress the button to the second stop. Release the button to draw excess sample into the tip. Press the button to the first stop to expel a precise volume of sample, leaving excess sample in the tip.

Staining the Cells
Stain whole blood samples following specific instructions in the appropriate reagent IFU. Use care to protect the tubes from direct light. Perform the procedure at room temperature (20°C–25°C).

9. RESULTS

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 × 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 tube foil pouch label and might vary from lot to lot.
10. PERFORMANCE CHARACTERISTICS

The performance of BD Trucount tubes was established for each relevant BD reagent that can be used with the tubes. See the respective reagent IFUs showing data for determining absolute counts using BD Trucount tubes.

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.

REFERENCES


