1. INTENDED USE

BD Oncomark™ CD7 FITC/CD33 PE is intended for in vitro flow cytometric immunophenotyping. This reagent combination can be used to study expression of the CD7 antigen in myeloid lineages. CD33 is useful in identifying myeloid lineage cells, CD7, normally expressed in T and natural killer (NK) cells, is frequently aberrantly expressed in acute myeloid leukemia (AML), and could be used in the diagnosis of these diseases. CD7/CD33 assays are used in the diagnosis of hematologic disorders.

2. COMPOSITION

CD7, clone M-T701, is derived from the hybridization of mouse P3-X63-Ag8.653 cells with spleen cells from BALB/c mice immunized with P-CLL and Jurkat cells. CD33, clone P67.6, is derived from the hybridization of mouse Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with FMY955 cells containing the CD33 gene.

CD7 and CD33 are each composed of mouse IgG1 heavy chains and kappa light chains.

This reagent is supplied as a combination of CD7 FITC and CD33 PE, in 1 mL of phosphate-buffered saline (PBS) containing gelatin and 0.1% sodium azide.

Antibody purity is as follows.

- FITC, PE: ≤20% free fluorophore at bottling, as measured by size-exclusion chromatography (SEC)
3. STORAGE AND HANDLING

The antibody 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.

4. REAGENTS OR MATERIALS REQUIRED BUT NOT PROVIDED

- Falcon® disposable 12 x 75-mm polystyrene test tubes or equivalent
- Micropipettor with tips
- Vortex mixer
- BD FACSM™ lysing solution (10X) (Catalog No. 349202). For dilution instructions and warnings, refer to the instructions for use (IFU).
- Centrifuge
- BD CellWASH™ (Catalog No. 349524) or a wash buffer of PBS with 0.1% sodium azide
- BD CellFIX™ (Catalog No. 340181) or 1% paraformaldehyde solution in PBS with 0.1% sodium azide Store at 2°C–8°C in amber glass for up to 1 week.
- Properly equipped cytometer. Flow cytometers must have laser excitation set at 488 nm and must be equipped to detect light scatter and the appropriate fluorescence, and have the appropriate analysis software installed for data acquisition and analysis. Refer to your instrument user's guide for instructions.

5. SPECIMEN(S)

BD Oncomark CD7 FITC/CD33 PE can be used for immunophenotyping by flow cytometry with peripheral blood and bone marrow aspirates collected in BD Vacutainer® EDTA tubes. Each type of specimen can have different storage conditions and limitations that should be considered prior to collection and analysis.9,10

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection11,12 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.

6. PROCEDURE

Viability of samples should be assessed and a cutoff value established. A cutoff value of at least 80% viable cells has been suggested.9

To avoid serum interference when using these reagents, it is necessary to pre-wash the sample using at least 25 volumes excess 1X PBS with 0.1% sodium azide (48 mL of 1X PBS with sodium azide per 2 mL of whole blood to be washed). Mix well. Pellet cells by centrifugation and resuspend in 1X PBS with 0.1% sodium azide to the original volume.

* Falcon is a registered trademark of Corning Incorporated.
1. Add 20 µL of BD Oncomark CD7/CD33 reagent to 100 µL of whole blood or pre-filtered bone marrow in a 12 x 75-mm tube.

2. Vortex gently and incubate for 15 to 20 minutes in the dark at room temperature (20°C–25°C).

3. Add 2 mL of 1X BD FACS lysing solution.

4. Vortex gently and incubate for 10 minutes in the dark at room temperature.

5. Centrifuge at 300 g for 5 minutes. Remove the supernatant.

6. Add 2 to 3 mL of BD CellWASH solution (or wash buffer) and centrifuge at 200 g for 5 minutes. Remove the supernatant.

7. Add 0.5 mL of BD CellFIX solution or 1% paraformaldehyde solution and mix thoroughly. Store at 2°C–8°C until analyzed.

Stained samples should be analyzed within 24 hours of staining.

Flow Cytometric Analysis

1. Set up the instrument as recommended by the manufacturer.

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.

2. Vortex the cells thoroughly at low speed to reduce aggregation before running them on the flow cytometer.

3. Run the sample on the flow cytometer. Verify that all populations are on scale. Optimize the instrument settings, if needed.

4. Acquire and analyze list-mode data using appropriate software.

5. On the appropriate plots, use the required combination of gates, regions, or quadrants to isolate the population of interest (Figure 1).

6. Determine antigen expression based on the sample negative population.

7. PERFORMANCE CHARACTERISTICS

Specificity

CD7 recognizes a human T- and NK-lymphocyte antigen, with a molecular weight of 40 kilodaltons (kDa). CD33 recognizes a human myelomonocytic antigen with a molecular weight of 67 kDa.

Antigen Distribution

The CD7 antigen is expressed throughout T-lymphocyte differentiation. It is present on 85%–90% of peripheral blood T lymphocytes. In normal individuals, CD7 reacts with all CD8+ lymphocytes, approximately 90% of CD4+ lymphocytes, and most NK lymphocytes. CD7 is weakly reactive with monocytes and does not
react with granulocytes or B lymphocytes. In leukemias, the CD7 antigen is present on the majority of T-lymphoid lineages.

The CD33 antigen is present on monocytes (bright) and granulocytes (dim). Granulocytes might be further subdivided into neutrophil, eosinophil, and basophil populations based on CD33 staining in combination with other cell-surface antigens. The CD33 antigen is expressed on blast cells in greater than 85% of AMLs, and it can be aberrantly expressed in acute lymphoblastic leukemia (ALL). Normal lymphocytes, platelets, and erythrocytes do not express the CD33 antigen.

8. LIMITATIONS

Use of therapeutic monoclonal antibodies in patient treatment can interfere with recognition of target antigens by this reagent. This should be considered when analyzing samples from patients treated in this fashion. BD Biosciences has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.

Use of this reagent combination for diagnostic evaluation of hematologic disorders should be performed in the context of a thorough immunophenotypic analysis including other relevant markers. Procedures using BD Oncomark reagents must adhere to the instructions for use for the specific instrument, software, and quality control procedures used by your laboratory.

Reagent performance data was collected typically with EDTA-treated specimens. Reagent performance can be affected by the use of other anticoagulants.

Samples with large numbers of nonviable cells can give erroneous results due to selective loss of populations and to increased nonspecific binding of antibodies to nonviable cells.

**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.

**TROUBLESHOOTING**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor resolution between debris and lymphocytes</td>
<td>Cell interaction with other cells and platelets</td>
<td>Prepare and stain another sample.</td>
</tr>
<tr>
<td>Rough handling of cell preparation</td>
<td>Cell interaction with other cells and platelets</td>
<td>Check cell viability; centrifuge cells at lower speed.</td>
</tr>
<tr>
<td>Inappropriate instrument settings</td>
<td>Insufficient reagent</td>
<td>Repeat staining with increased amount of antibody.</td>
</tr>
<tr>
<td>Staining dim or fading</td>
<td>Insufficient reagent</td>
<td>Repeat staining with fresh sample; analyze promptly.</td>
</tr>
<tr>
<td>Few or no cells</td>
<td>Cells not analyzed within 24 hours of staining</td>
<td>Repeat staining with fresh sample; analyze promptly.</td>
</tr>
<tr>
<td>Few or no cells</td>
<td>Cell concentration too low</td>
<td>Resume fresh sample at a higher concentration; repeat staining and analysis.</td>
</tr>
<tr>
<td>Few or no cells</td>
<td>Cytemeter malfunctioning</td>
<td>Troubleshoot instrument.</td>
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REFERENCES


