1. INTENDED USE

BD Oncomark™ CD2 FITC/CD7 PE+/CD3 PerCP-Cy5.5* is intended for in vitro flow cytometric immunophenotyping of normal T-cell development and aberrant expression of the T-cell surface markers in various T-cell neoplasias. While the antigens recognized by these reagents are present on normal T cells, one or more of them is frequently expressed at different levels, or possibly absent, in abnormal T cells. One or more of the antigens detected by these antibodies might be aberrantly expressed in myeloid leukemia. CD2/CD7/CD3 assays are used in the diagnosis of hematologic disorders.

2. COMPOSITION

CD2, clone S5.2, is derived from the hybridization of mouse Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with T lymphocytes activated by mixed lymphocyte culture.

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.

CD3, clone SK7, is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with human thymocytes.

CD2 is composed of mouse IgG2a heavy chains and kappa light chains. CD7 and CD3 are each composed of mouse IgG1 heavy chains and kappa light chains.

* Patents—PE: US 4,520,110; 4,859,582; 5,055,556; Europe 76,695; Canada 1,179,942
PeCP: US 4,876,190
Cy5.5: US 5,268,486; 5,486,616; 5,569,587; 5,569,766; 5,627,027
This reagent is supplied as a combination of CD2 FITC, CD7 PE, and CD3 PerCp-Cy5.5 in 1 mL of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) and 0.1% sodium azide.

**WARNING** Sodium azide is harmful if swallowed (R22). Keep out of reach of children (S2). Keep away from food, drink, and animal feedingstuff (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.

Antibody purity is as follows.

- FITC, PE, PerCp-Cy5.5: ≤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° to 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

- BD Falcon™ disposable 12 x 75-mm capped polystyrene test tubes (BD Catalog No. 352058) or equivalent.
- Micropipettor with tips (BD Electronic Pipette, BD Catalog No. 343246 [US], 349444 [Europe])
- Vortex mixer
- BD FACS™ lysing solution* (10X) (BD Catalog No. 349202)

For dilution instructions and warnings, refer to the product insert.
- Centrifuge
- BD CellWASH™ solution (BD Catalog No. 349524) or a wash buffer of phosphate-buffered saline (PBS) with 0.1% sodium azide
- BD CellFIX™ solution (BD Catalog No. 340181) or 1% paraformaldehyde solution in PBS with 0.1% sodium azide

Store at 2° to 8°C in amber glass for up to 1 week.

**WARNING** Formaldehyde is harmful by inhalation, in contact with skin, and toxic if swallowed (R20/21, 25). It is irritating to eyes and skin (R36/38). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S26). Exposure can cause cancer. Contact with acids liberates very toxic gas (R32). Possible risks of irreversible effects (R68). Can cause sensitization by skin contact (R43). When using do not eat or drink (S20).

- 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.

* US Patent Nos. 4,654,312; 4,902,613; 5,098,849
Refer to your instrument user’s guide for instructions.

5. SPECIMEN(S)
BD Oncomark CD2 FITC/CD7 PE/CD3 PerCP-Cy5.5 can be used for immunophenotyping by flow cytometry with peripheral blood and bone marrow aspirates collected in EDTA (eg, BD Vacutainer™ tubes). Each type of specimen can have different storage conditions and limitations that should be considered prior to collection and analysis.\textsuperscript{13,14}

\textbf{WARNING} All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection\textsuperscript{15,16} and dispose of with proper precautions in accordance with federal, state, and local regulations. Never pipette by mouth. Wear suitable protective clothing 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.\textsuperscript{13}

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% azide (ie, 48 mL of 1X PBS with azide per 2 mL whole blood to be washed). Mix well. Pellet cells by centrifugation and resuspend in 1X PBS with 0.1% azide to the original volume.

1. Add 20 µL of BD Oncomark CD2/CD7/CD3 reagent to 100 µL of whole blood or prefiltered bone marrow in a 12 x 75-mm tube.

2. Vortex gently and incubate 15 to 20 minutes in the dark at room temperature (20° to 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 x g for 5 minutes. Remove the supernatant.

6. Add 2 to 3 mL of BD CellWASH solution (or wash buffer) and centrifuge at 200 x 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° to 8°C until analyzed. Stained samples should be analyzed within 24 hours of staining.

\textbf{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.\textsuperscript{17}

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).

Figure 1 Dot plots displaying region R1 and quadrants

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

7. PERFORMANCE CHARACTERISTICS

Specificity
CD2 recognizes a human lymphocyte antigen, Mr 45 to 50 kilodaltons (kd). 18
CD7 recognizes a human T- and natural killer (NK)-lymphocyte antigen, Mr 40 kd. 19,20
CD3 reacts with the epsilon chain of the CD3 antigen/T-cell antigen receptor (TCR) complex. 21 The antigen recognized by CD3 antibodies is noncovalently associated with either αβ or γδ TCR (70 to 90 kd). 22

Antigen Distribution
The CD2 antigen is present on approximately 75% of normal peripheral blood lymphocytes and 95% to 99% of thymocytes. 23 The CD2 antibody reacts with essentially all T lymphocytes and with a subset of NK lymphocytes. 24
The CD7 antigen is expressed throughout T-lymphocyte differentiation. 20 It is present on 85% to 90% of peripheral blood T lymphocytes. 20 In normal individuals, CD7 reacts with all CD8+ lymphocytes, approximately 90% of CD4+ lymphocytes, and most NK lymphocytes. 20 CD7 is weakly reactive with monocytes and does not react with granulocytes 19 or B lymphocytes. 20
In leukemias, the CD7 antigen is present on the majority of T-lymphoid lineages. 25
The CD3 antigen is present on 61% to 85% of normal peripheral blood lymphocytes. 26

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 the BD Oncomark reagents must adhere to the instructions for use for the specific instrument, soft-
ware, and quality control procedures used by your laboratory.

Reagent data performance 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**

The product sold hereunder is warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, that extend beyond the description on the label of the product. 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, personal injury, or economic loss caused by the product.

<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>Poor resolution between debris and lymphocytes</td>
<td>Rough handling of cell preparation</td>
<td>Check cell viability; centrifuge cells at lower speed.</td>
</tr>
<tr>
<td>Poor resolution between debris and lymphocytes</td>
<td>Inappropriate instrument settings</td>
<td>Follow proper instrument setup procedures; optimize instrument settings as required.</td>
</tr>
<tr>
<td>Staining dim or fading</td>
<td>Cell concentration too high at staining step</td>
<td>Check and adjust cell concentration or sample volume; stain with fresh sample.</td>
</tr>
<tr>
<td>Staining dim or fading</td>
<td>Insufficient reagent</td>
<td>Repeat staining with increased amount of antibody.</td>
</tr>
<tr>
<td>Staining dim or fading</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>Resuspend fresh sample at a higher concentration; repeat staining and analysis.</td>
</tr>
<tr>
<td>Few or no cells</td>
<td>Cytometer malfunctioning</td>
<td>Troubleshoot instrument.</td>
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REFERENCES


2. Porwit-MacDonald A. Multiparameter flow cytometry detection of minimal residual disease in T-cell acute lymphoblastic leukemia. Presented at the International Symposium on Minimal Residual Disease From methodological problems to clinical goals; October 31-November 1, 1997; Salamanca, Spain.


