1. INTENDED USE
BD Oncomark™ CD15 FITC/CD34 PE is intended for in vitro flow cytometric immunophenotyping. CD15/CD34 assays are used in the diagnosis of hematologic disorders.1-5

2. COMPOSITION
CD15, clone MMA, is derived from the hybridization of mouse P3-X63-Ag8.653 myeloma cells with spleen cells from BALB/c mice immunized with the U-937 histiocytic cell line.

CD34 (Anti–HPCA*-2), clone 8G12, is derived from the hybridization of mouse Sp2/0-Ag14 cells with spleen cells of BALB/c mice immunized with the human cell line KG-1a.6

CD15 is composed of mouse IgM heavy chains and kappa light chains. CD34 (Anti–HPCA-2) is composed of mouse IgG1 heavy chains and kappa light chains.

This reagent is supplied as a combination of CD15 FITC and CD34 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 FACS™ 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 CD15 FITC/CD34 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.7,8

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection9,10 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.7

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.

1. Add 20 µL of BD Oncomark CD15/CD34 reagent to 100 µL of whole blood or prefiltered 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).

* Falcon is a registered trademark of Corning Incorporated.
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 300g for 5 minutes. Remove the supernatant.
6. Add 2 to 3 mL of BD CellWASH solution (or wash buffer) and centrifuge at 200g 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
CD15 recognizes the human myelomonocytic antigen lacto-N-fucopentose III.

CD34 recognizes a single-chain transmembrane glycoprotein, 105 to 120 kilodaltons (kDa). The antigen is associated with human hematopoietic progenitor cells and is a differentiation stage–specific leucocyte antigen. Clone 8G12 recognizes the class 3 epitope.

Anti–HPCA-1 and Anti–HPCA-2 (clone 8G12) recognize two distinct CD34 epitopes; at least three epitopes have been identified. Clone 8G12 recognizes the class 3 epitope.
Antigen Distribution
The CD15 antigen, present on greater than 95% of mature peripheral blood eosinophils and neutrophils, is present at low density on circulating monocytes.18 The CD34 antigen is present on immature hematopoietic precursor cells and all hematopoietic colony-forming cells in bone marrow and blood.14,17 The CD34 antigen is also found on capillary endothelial cells and approximately 1% of human thymocytes.14,19 Normal peripheral blood lymphocytes, monocytes, granulocytes, and platelets do not express the CD34 antigen.6,14,17 CD34 antigen density is highest on early hematopoietic progenitor cells and decreases as cells mature. The antigen is absent on fully differentiated hematopoietic cells.14,20,21

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.

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.

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></td>
<td>Check cell viability; centrifuge cells at lower speed.</td>
</tr>
<tr>
<td>Inappropriate instrument settings</td>
<td></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>
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<tr>
<td>Insufficient reagent</td>
<td></td>
<td>Repeat staining with increased amount of antibody.</td>
</tr>
<tr>
<td>Cells not analyzed within 24 hours of staining</td>
<td></td>
<td>Repeat staining with fresh sample; analyze promptly.</td>
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REFERENCES


