1. INTENDED USE

BD Oncomark™ CD38 FITC/CD56 PE/CD19 PerCP-Cy™5.5* is intended for in vitro flow cytometric immunophenotyping of normal and abnormal plasma cells. Plasma cells are usually CD38 bright. The CD56 versus CD19 phenotypes of the CD38 bright cells might be different in various types of plasma cell diseases. CD38/CD56/CD19 assays are used in the diagnosis of hematologic disorders.

2. COMPOSITION

CD38, clone HB7, is derived from the hybridization of mouse P3-X63-Ag8.653 myeloma cells with spleen cells from BALB/c mice immunized with the BJAB cell line.

CD56, clone NCAM16.2, is derived from the hybridization of mouse P3-X63-Ag8.653 myeloma cells with lymph nodes from BALB/c mice immunized with immunoaffinity-enriched adult human brain neural cell adhesion molecules (NCAMs).

CD19, clone SJ25C1, is derived from the hybridization of mouse Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with NALM-1 + NALM-16 cells.

CD38 and CD19 are each composed of mouse IgG1 heavy chains and kappa light chains.

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chains. CD56 is composed of mouse IgG2b heavy chains and kappa light chains.

This reagent is supplied as a combination of CD38 FITC, CD56 PE, and CD19 PerCP-Cy5.5 in 1 mL of phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) and 0.1% sodium azide.

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°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 FACSTM 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 CD38 FITC/CD56 PE/CD19 PerCP-Cy5.5 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.11,12

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection13,14 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.

† Falcon is a registered trademark of Corning Incorporated.
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{11}

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 CD38/CD56/CD19 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).
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.
2. Vortex the cells thoroughly at low speed to reduce aggregation before running them on the flow cytometer.\textsuperscript{15}
3. Run the sample on the flow cytometer.
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).

![Flow Cytometric Analysis Figure 1](image-url)
6. Determine antigen expression based on the sample negative population.

7. PERFORMANCE CHARACTERISTICS

Specificity
CD38 recognizes an integral membrane glycoprotein, with a molecular weight of 45 kilodaltons (kDa), with a protein core of 35 kDa.\(^{16}\)

CD56 (NCAM16.2) recognizes an extracellular immunoglobulin-like domain common to three molecular weight forms (120, 140, and 180 kDa) of NCAM.\(^{17-19}\)

CD19 (SJ25C1) recognizes a 90-kDa antigen that is present on human B lymphocytes.\(^{10,20}\)

Antigen Distribution

The CD38 antigen is expressed on essentially all pre-B lymphocytes, plasma cells, and thymocytes.\(^{16}\) It is also present on activated T lymphocytes, natural killer (NK) lymphocytes, and myeloblasts.\(^{5,8,16}\)

The antigen is expressed during the early stages of T- and B-lymphocyte differentiation, is lost during the intermediate stages of maturation, and then reappears during the final stages of maturation.\(^{8,16}\) It is also expressed in T- and B-acute lymphoblastic leukemia (T- and B-ALL), Burkitt’s lymphoma, multiple myeloma, and acute myeloid leukemia (AML).\(^{21,22}\)

The CD56 antigen is present on approximately 10% to 25% of peripheral blood lymphocytes.\(^{23}\) It is present on essentially all resting and activated CD16+ NK lymphocytes and approximately 5% of CD3+ peripheral blood lymphocytes.\(^{23}\)

The CD19 antigen is present on approximately 7% to 23% of human peripheral blood lymphocytes\(^ {24}\) and on splenocytes.\(^ {25}\) The CD19 antigen is present on human B lymphocytes at most stages of maturation.\(^ {10,26}\) CD19 does not react with resting or activated T lymphocytes, granulocytes, or monocytes.\(^ {10,26}\)

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 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 HEREBY 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>Check cell viability; centrifuge cells at lower speed.</td>
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<tr>
<td>Inappropriate instrument settings</td>
<td>Follow proper instrument setup procedures; optimize instrument settings as required.</td>
<td></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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<td>Insufficient reagent</td>
<td>Repeat staining with increased amount of antibody.</td>
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<tr>
<td>Cells not analyzed within 24 hours of staining</td>
<td>Repeat staining with fresh sample; analyze promptly.</td>
<td></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>
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<tr>
<td>Cytometer malfunctioning</td>
<td>Troubleshoot instrument.</td>
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REFERENCES


