1. INTENDED USE
BD Oncomark™ CD45 FITC/Anti–glycophorin A PE/CD41a PerCP-Cy™5.5* is intended for in vitro flow cytometric immunophenotyping of the development in the erythroid, leucocytic, and megakaryocytic cell lineages in peripheral blood and bone marrow. Megakaryocytes co-express the platelet-specific glycoprotein gpIIb/IIIa (CD41a) and CD45 throughout their differentiation.1 In contrast, glycophorin A expression is present during erythroid cell development, with a gradual decrease in CD45 intensity during erythroid maturation. Using this approach, erythroid and lymphoid cells can be clearly distinguished based upon their antigenic expression.2 CD45/Anti–glycophorin A/CD41a assays are used in the diagnosis of hematologic disorders.1,2

2. COMPOSITION
CD45, clone 2D1, is derived from the hybridization of mouse NS-1 myeloma cells with spleen cells from BALB/c mice immunized with human peripheral blood mononuclear cells (PBMCs).
Anti–glycophorin A is clone GAR-2 (HIR-2).

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CD41a, clone HIP8, is derived from the hybridization of mouse P3X63 myeloma cells with spleen cells from BALB/c mice immunized with purified platelet membrane glycoproteins.

CD45 and CD41a are each composed of mouse IgG1 heavy chains and kappa light chains. Anti-glycophorin A is composed of mouse IgG2b heavy chains and kappa light chains.

This reagent is supplied as a combination of CD45 FITC, Anti–glycophorin A PE, and CD41a 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

- 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 CD45 FITC/Anti–glycophorin A PE/CD41a 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.3,4

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection5,6 and dispose of with proper precautions in

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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.3

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.

CAUTION The Anti–glycophorin A antibody concentration is appropriate for staining samples containing mature red blood cells. If these cells are removed from the sample before staining, the remaining nucleated, immature red cells will stain too brightly. If this occurs, it is necessary to reoptimize the reagent concentration or instrument settings.

1. Add 20 µL of BD Oncomark CD45/Anti–glycophorin A/CD41a 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. 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.7
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
CD45 (Anti–HLe-1) recognizes human leucocyte antigens, with molecular weights from 180 to 220 kilodaltons (kDa), that are members of the T200 family.\(^8\)

Anti–glycophorin A reacts with a major sialoglycoprotein present on the human erythrocyte membrane.\(^9\) Glycophorin A is a transmembrane dimeric complex of 31 kDa with its carboxyterminal ends extending into the cytoplasm of the red cell.\(^10\)

CD41a recognizes the calcium-dependent gpIIb/IIIa complex, 140 kDa.\(^11\)

Antigen Distribution
The CD45 antigen is present on all human leucocytes, including lymphocytes, monocytes, granulocytes, eosinophils, and basophils in peripheral blood and has a role in signal transduction, modifying signals from other surface molecules.\(^8\) The CD45 antibody has been reported to react weakly with mature circulating erythrocytes and platelets.\(^8,12\)

Glycophorin A is expressed on human red blood cells, normoblasts, and erythroid precursor cells.\(^13\) It is also found on erythroid leukemias and some CD34\(^+\) M7 megakaryoblastic leukemias.\(^14-16\) Mature non-nucleated red blood cells are characteristically glycophorin A–positive but CD45 negative.\(^17\) Glycophorin A was reported to serve as a receptor for several infectious disease agents.\(^18-20\)

The CD41a antigen (gpIIb/IIIa complex) is found on platelets and platelet precursors. It acts as a receptor for fibrinogen, von Willebrand factor (vWF), fibronectin, and vitronectin, and it mediates platelet adhesion and aggregation.\(^21-23\)

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

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<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
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<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>
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<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>
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REFERENCES


