CD41a (HIP8)
Monoclonal mouse anti-human reagent for identification of cells expressing CD41a antigen

<table>
<thead>
<tr>
<th>Form</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC</td>
<td>333147</td>
</tr>
<tr>
<td>PerCP-Cy5.5</td>
<td>333148</td>
</tr>
</tbody>
</table>

1. INTENDED USE
CD41a is intended for in vitro diagnostic use in the identification of cells expressing CD41a antigen, using a BD FACSTM brand flow cytometer.

The flow cytometer must be equipped to detect light scatter and the appropriate fluorescence, and be equipped with appropriate analysis software (such as BD CellQuestTM or BD LYSYS™ II software) for data acquisition and analysis. Refer to your instrument user’s guide for instructions.

Applications
Expression of CD41a antigen in the characterization of hematologic neoplasia1-3

2. COMPOSITION
CD41a, clone HIP8, is derived from the hybridization of mouse P3-X63-Ag8.653 myeloma cells with spleen cells from BALB/c mice immunized with purified platelet membrane glycoproteins. CD41a is composed of mouse IgG1 heavy chains and kappa light chains.

Each reagent is supplied in phosphate-buffered saline (PBS) containing gelatin and 0.1% sodium azide. Concentrations are listed in Table 1.

Table 1 Bottling concentrations

<table>
<thead>
<tr>
<th>Form</th>
<th>Amount provided</th>
<th>Conc(^a) (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FITC</td>
<td>25 µg in 1.0 mL of PBS</td>
<td>25</td>
</tr>
<tr>
<td>PerCP-Cy5.5</td>
<td>25 µg in 1.0 mL of PBS</td>
<td>25</td>
</tr>
</tbody>
</table>

a. Conc = concentration
Antibody purity is as follows:

- **FITC**: ≤5% free fluorophore at bottling, as measured by size-exclusion chromatography (SEC)
- **PerCP-Cy5.5**: ≤20% free fluorophore at bottling, as measured by 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** solution (Catalog No. 349524) or a wash buffer of PBS with 0.1% sodium azide
- **BD CellFIX** solution (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.
- **BD FACS brand flow cytometer.** Refer to the appropriate instrument user’s guide for information.

5. SPECIMEN(S)

Reagents can be used for immunophenotyping by flow cytometry with a variety of specimen types, including peripheral blood, bone marrow aspirates or biopsies, and other body fluids or tissues. Each type of specimen can have different storage conditions and limitations that should be considered prior to collection and analysis.

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

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

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* Falcon® is a registered trademark of Corning Incorporated.
6. PROCEDURE

1. Add the appropriate volume of CD41a fluorochrome-conjugated monoclonal antibody to 100 µL of whole blood in a 12 x 75-mm tube. Refer to the appropriate vial label for volume.

2. Vortex gently and incubate for 15 to 30 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. We recommend analyzing within 24 hours of staining.

Analytical Results

Abnormal numbers of cells expressing this antigen or aberrant expression levels of the antigen can be expected in some disease states. It is important to understand the normal expression pattern for this antigen and its relationship to expression of other relevant antigens in order to perform appropriate analysis.

Flow Cytometry

Vortex the cells thoroughly at low speed to reduce aggregation before running them on the flow cytometer. Acquire and analyze list-mode data using appropriate software. Before acquiring samples, adjust the threshold to minimize debris and ensure populations of interest are included. Figure 1 displays representative data performed on fixed platelets and gated on platelets. Laser excitation is at 488 nm.

**Figure 1** Representative data analyzed with a BD FACS brand flow cytometer

<table>
<thead>
<tr>
<th>No.</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>2</td>
<td>Platelets</td>
</tr>
</tbody>
</table>
Internal Quality Control
We recommend using BD Calibrite™ beads and BD FACScComp™ software to set photomultiplier tube (PMT) voltages, fluorescence compensation, and to check instrument sensitivity prior to use. Refer to the BD Calibrite Beads IFU and the BD FACScComp Software User’s Guide.

We recommend running 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.9

7. PERFORMANCE CHARACTERISTICS

Specificity
CD41a recognizes the calcium-dependent gpIIb/IIIa complex with a molecular weight of 140 kilodaltons (kDa).10

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.11-13

Sensitivity
Sensitivity is defined as resolution of the CD41a+ population from the CD41a− population. Sensitivity was measured by evaluating a range of antibody concentrations. Each concentration of reagent was tested on whole blood. The separation of CD41a+ from CD41a− was determined for each sample and averaged within each concentration. The bottled antibody concentration for each reagent provided optimum sensitivity in resolving the CD41a+ cells from the negative. See Table 1.

Reproducibility
CD41a was submitted to the Fourth International Workshop and Conference on Human Leucocyte Differentiation Antigens. Participating laboratories evaluated clone HIP8 as part of a blind panel of antibodies and reported consistent results.10

Repeatability
To determine the repeatability of staining with each reagent, samples were stained with multiple lots of reagents. The different samples used in the evaluation provided an average mean fluorescence intensity (MFI) value as shown in Table 2. For each sample, two different lots of reagents generated a pair of results. Individual standard deviations (SDs) were determined from the paired results for each sample. Individual SDs were combined to derive a pooled SD for each reagent that provides an estimate of within-sample repeatability.

| Table 2 Repeatability of MFI of CD41a+ cells across different lots and across multiple donors (N) |
|-------------------------------------------------|----------|---------|---------|
| FITC                                            | Na = 6  | 302.18  | 8.77    | 2.9     |
| PerCP-Cy5.5                                     | Na = 3  | 433.3   | 35.67   | 8.23    |

a. Na = number of samples  
b. CV = coefficient of variation

8. LIMITATIONS
Conjugates with brighter fluorochromes (PE, APC) will give greater separation than those with other dyes (FITC, PerCP). When populations overlap, calculation of the percentage of cells positive for the marker can be affected by the choice of fluorochrome.
Use of 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.

Single reagents can provide only limited information in the analysis of leukemias and lymphomas. Using combinations of reagents can provide more information than using the reagents individually. Multicolor analysis using relevant combinations of reagents is highly recommended.5

As reagents can be used in different combinations, laboratories need to become familiar with the properties of each antibody in conjunction with other markers in normal and abnormal samples.

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

**WARRANTY**

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### 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>
</tr>
<tr>
<td></td>
<td>Insufficient reagent</td>
<td>Repeat staining with increased amount of antibody.</td>
</tr>
<tr>
<td></td>
<td>Cells not analyzed within 24 hours of staining</td>
<td>Repeat staining with fresh sample; analyze promptly.</td>
</tr>
<tr>
<td></td>
<td>Improper medium preparation (sodium azide omitted)</td>
<td>Use sodium azide in staining medium and washing steps.</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>Cytometer malfunctioning</td>
<td></td>
<td>Troubleshoot instrument.</td>
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</tbody>
</table>

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### REFERENCES


