CD14 (MϕP9)

Monoclonal mouse anti-human reagent for identification of cells expressing CD14 antigen

Form Catalog No.
FITC 345784
PE 345785
PerCP 345786
APC 345787
APC-Cy7 333951

1. INTENDED USE

CD14 is intended for in vitro diagnostic use in the identification of cells expressing CD14 antigen, using a BD FACS™ 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 CellQuest™ or BD LYSYS™ II software) for data acquisition and analysis. Refer to your instrument user’s guide for instructions.

Applications

Expression of CD14 in the characterization of hematologic neoplasia

2. COMPOSITION

CD14, clone MϕP9, is derived from hybridization of mouse Sp2/0 myeloma cells with spleen cells from BALB/c mice immunized with peripheral blood monocytes from a patient with rheumatoid arthritis. CD14 is composed of mouse IgG2b 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>
<thead>
<tr>
<th>Table 1 Bottling concentrations</th>
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Antibody purity is as follows.

- FITC: ≤5% free fluorophore at bottling, as measured by size-exclusion chromatography (SEC)
- PE, PerCP, APC, APC-Cy7: ≤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 FACS™ lyzing 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.
- 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.4,10

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.4

WARNING All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection11,12 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**

1. Add the appropriate volume of CD14 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.

**CAUTION** Some APC-Cy7 conjugates show changes in their emission spectra with prolonged exposure to paraformaldehyde. For overnight storage of stained cells, wash and resuspend in buffer without paraformaldehyde after 1 hour of fixation.

**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 whole blood and gated on monocytes. Laser excitation is at 488 nm and 635 nm.

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

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**Internal Quality Control**

We recommend using BD Calibrite™ beads and BD FACSComp™ 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 FACSComp 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.\textsuperscript{14}

7. PERFORMANCE CHARACTERISTICS

Specificity
The CD14 antigen is present on the majority of normal peripheral blood monocytes.\textsuperscript{15} CD14 has weak reactivity with peripheral blood granulocytes.\textsuperscript{16} CD14 recognizes a human monocyte/macrophage antigen with a molecular weight of 55 kilodaltons (kDa).\textsuperscript{17}

Sensitivity
Sensitivity is defined as resolution of the CD14\textsuperscript{+} population from the CD14\textsuperscript{−} population. Sensitivity was measured by evaluating a range of antibody concentrations. Each concentration of reagent was tested on whole blood. The separation of CD14\textsuperscript{+} from CD14\textsuperscript{−} was determined for each sample and averaged within each concentration. The bottled antibody concentration for each reagent provided optimum sensitivity in resolving the CD14\textsuperscript{+} cells from the negative. See Table 1.

Reproducibility
CD14 was submitted to the First International Workshop and Conference on Human Leucocyte Differentiation Antigens. Participating laboratories evaluated clone M_
ϕP9 as part of a blind panel of antibodies and reported consistent results.\textsuperscript{18}

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.

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.\textsuperscript{10}

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**

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.

**TROUBLESHOOTING**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
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<tbody>
<tr>
<td>Few or no cells</td>
<td>Cell concentration too low</td>
<td>Resuspend sample at a higher concentration; repeat staining and analysis.</td>
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<td>Cytometer malfunctioning</td>
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**REFERENCES**


