

BD Lineage Cocktail 1 (CD3, CD14, CD16, CD19, CD20, CD56) **Monoclonal Antibodies Detecting Human Antigens**

Form	Catalog number
FITC	340546

Product availability varies by region. Contact BD Biosciences Customer Support or your local sales representative for information.

Research Applications

Research applications include studies of:

- Dendritic cell subsets and basophils in peripheral blood¹⁻⁸

Description

Specificity

The Lineage Cocktail 1 (lin 1) contains antibody clones that, in combination, stain lymphocytes, monocytes, eosinophils, and neutrophils. It includes CD3, CD14, CD16, CD19, CD20, and CD56. Peripheral blood dendritic cells¹⁻⁵ and basophils⁶⁻⁸ can be distinguished from other leucocytes by their lack of staining with lin 1.

Antigen distribution

The CD3 antigen is expressed on all T lymphocytes.

The CD14 antigen is expressed on monocytes, macrophages, neutrophils, and eosinophils.

The CD16 antigen is expressed on resting natural killer (NK) lymphocytes, macrophages, cultured monocytes, and neutrophils.

The CD19 and CD20 antigens are expressed on B lymphocytes.

The CD56 antigen is expressed on activated and resting NK lymphocytes.

Composition

The following reagents are each composed of mouse IgG₁ heavy chains and kappa light chains:

- CD3, clone SK7
- CD16, clone 3G8
- CD19, clone SJ25C1
- CD20, clone L27

The following reagents are each composed of mouse IgG_{2b} heavy chains and kappa light chains:

- CD14, clone MfP9
- CD56, clone NCAM16.2

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Product configuration

The following reagent is supplied in buffer containing a stabilizer and a preservative.

Form	Number of tests	Volume per test (μL)	Amount provided (μg)	Total volume (mL)	Stabilizer	Preservative
FITC	50	20	140.6	1.0	Gelatin	0.1% Sodium azide

Procedure

Go to our website (bdbiosciences.com) or contact your local BD representative for the lyse/wash protocol for direct immunofluorescence.

For a 4-color assay: Add 20 μL lin 1 FITC, 5 μL CD123 (Anti-IL-3Rα) PE (Cat. No. 340545), 10 μL Anti-HLA-DR PerCP (Cat. No. 347364), and 5 μL CD11c APC (Cat. No. 340544) to a test tube. Then add 100 μL of whole blood. Mix gently and incubate for 15 to 30 minutes in the dark at room temperature (20–25 °C). Add 2 mL 1X BD FACS™ Lysing Solution (Cat. No. 349202) at room temperature, mix gently and incubate for no more than 10 minutes at room temperature in the dark. Centrifuge at 300 x g for 5 minutes. Wash cells with 1 mL of 1X PBS with 0.1% sodium azide, add 0.3 mL of 1% paraformaldehyde, mix, and analyze. Refer to the *BD FACS™ Lysing Solution* package insert.

NOTE The 3-color assay uses two staining combinations to identify CD123⁺ and CD11c⁺ dendritic cell subsets. Use 5 μL CD123, 10 μL HLA-DR PerCP, and 5 μL CD11c PE for this application.

Representative Data

Flow cytometric analysis was performed on whole blood stained with the indicated conjugated antibody. Laser excitation was at 488 nm and 635 nm. Representative data analyzed with aBD flow cytometer is shown in the following plots.

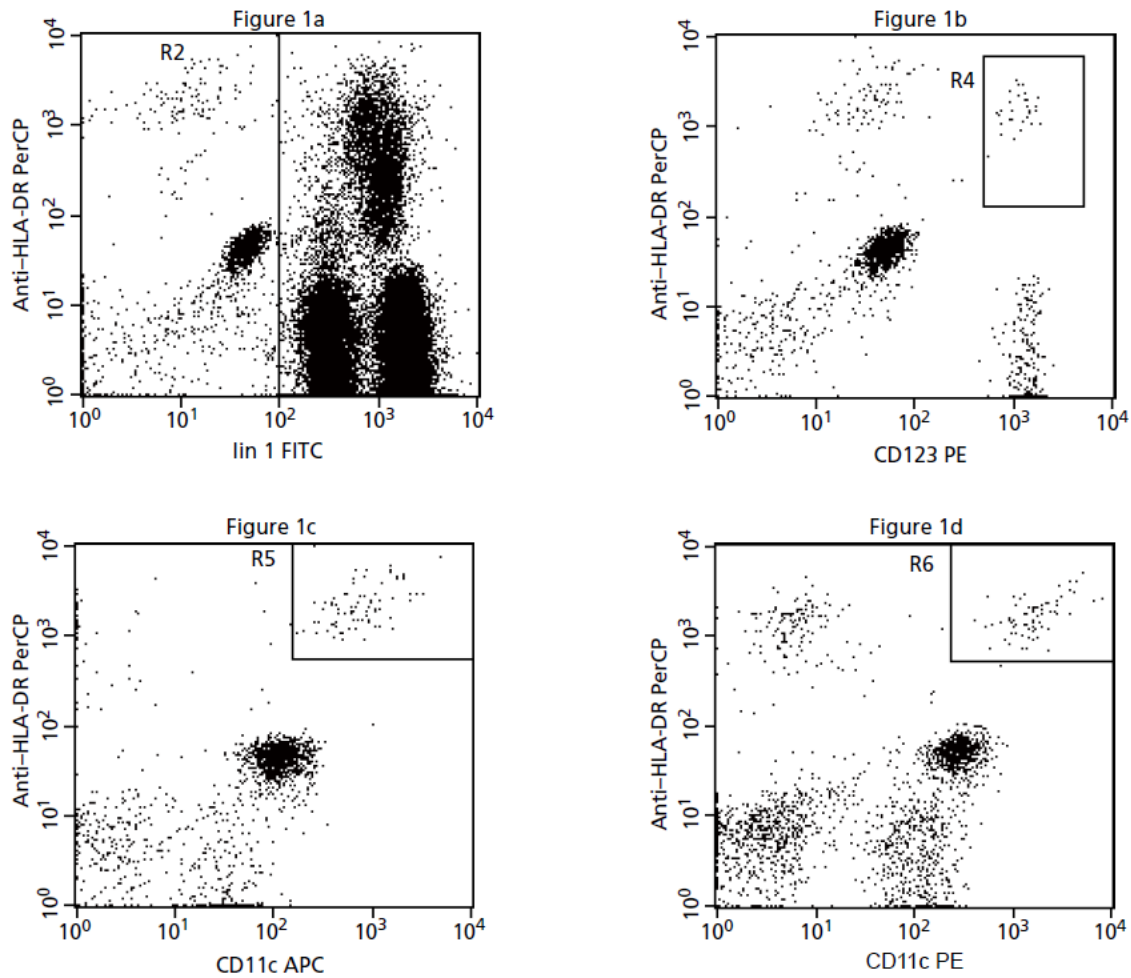
The Lineage Cocktail 1 (lin 1) can be used in a 3- or 4-color assay to identify dendritic cells in peripheral blood. Dendritic cells express high levels of HLA-DR and lack CD3, CD14, CD16, CD19, CD20, and CD56.^{1-5,9} Gated on lin 1 dim/negative events, two dendritic cell subsets can be identified based on their high HLA-DR expression and their high CD123¹ or CD11c⁹⁻¹¹ antigen distribution. This is illustrated in Figure 1b, Figure 1c, and Figure 1d (page 3). The upper region in Figure 1b shows the CD123⁺ (Anti-IL-3Rα⁺) dendritic cell subset. Basophils lack the HLA-DR antigen but overlap dendritic cells in expression of CD123⁺ (lower right population in Figure 1b).^{1,7,8,12} The regions in Figure 1c (4-color assay) and Figure 1d on page 3 (3-color assay) show the CD11c⁺ dendritic cell subset.

NOTE Additional gating is recommended in aged or abnormal samples. For information and more detailed gating strategies, refer to the BD application note, *Peripheral Blood Dendritic Cells Revealed by Flow Cytometry*.

3-Color Assay: The 3-color assay uses two staining combinations to identify CD123⁺ and CD11c⁺ dendritic cell subsets: lin 1 FITC, CD123 PE, and Anti-HLA-DR PerCP; and lin 1 FITC, CD11c PE, and Anti-HLA-DR PerCP (Figure 1a, Figure 1b, and Figure 1d). Performed on lysed whole blood. Laser excitation is at 488 nm; 50,000 events were acquired with an FSC threshold set within an FSC vs SSC dot plot.

4-Color Assay: The 4-color assay requires one staining combination to identify CD123⁺ and CD11c⁺ dendritic cell subsets: lin 1 FITC, CD123 PE, Anti-HLA-DR PerCP, and CD11c APC (Figure 1a, Figure 1b, and Figure 1c). Acquire a minimum of 50,000 events with an FSC threshold. Performed on lysed whole blood. Laser excitation is at 488 nm and 635 nm; 50,000 events were acquired with an FSC threshold set within an FSC vs SSC dot plot.

Figure 1 Representative data analyzed with a BD flow cytometer



Handling and Storage

Store vials at 2–8 °C. Conjugated forms should not be frozen. Protect from exposure to light. Each reagent is stable until the expiration date shown on the bottle label when stored as directed.

Warning

All biological specimens and materials coming in contact with them are considered biohazards. Handle as if capable of transmitting infection^{13,14} 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.

Go to regdocs.bd.com to download the Safety Data Sheet.

Characterization

To ensure consistently high-quality reagents, each lot of antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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.

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Patents and Trademarks

For US patents that may apply, see bd.com/patents.

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