

## PRODUCT DESCRIPTION

This kit contains four conjugated antibodies (and corresponding isotype controls) that can be used for the single-step staining of human myeloid dendritic cells (mDCs) (1-4).

## MATERIALS PROVIDED & STORAGE

Store the unopened kit at 2-8 °C **in the dark**. Refer to the kit label for date of expiration.

PART	PART #	DESCRIPTION
Positive Markers	967212	250 µL of BDCA-3/CD141-PE Mouse IgG <sub>1</sub> (Clone 501733)
	967213	250 µL of BDCA-1/CD1c-APC Goat IgG
	967214	250 µL of CD11c-CFS Mouse IgG <sub>1</sub> (Clone ICRF 3.9)
	967215	250 µL of CD16/Fcγ RIII-PerCP Mouse IgG <sub>2A</sub> (Clone 245536)
Isotype Controls	965666	250 µL of Mouse IgG <sub>1</sub> -PE
	967140	250 µL of Goat IgG-APC
	965668	250 µL of Mouse IgG <sub>1</sub> -CFS
	967223	250 µL of Mouse IgG <sub>2A</sub> -PerCP
Staining Buffer	895027	100 mL of 1X Staining Buffer

## PRECAUTION

The Staining Buffer in this kit contains 0.09% sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

## REFERENCES

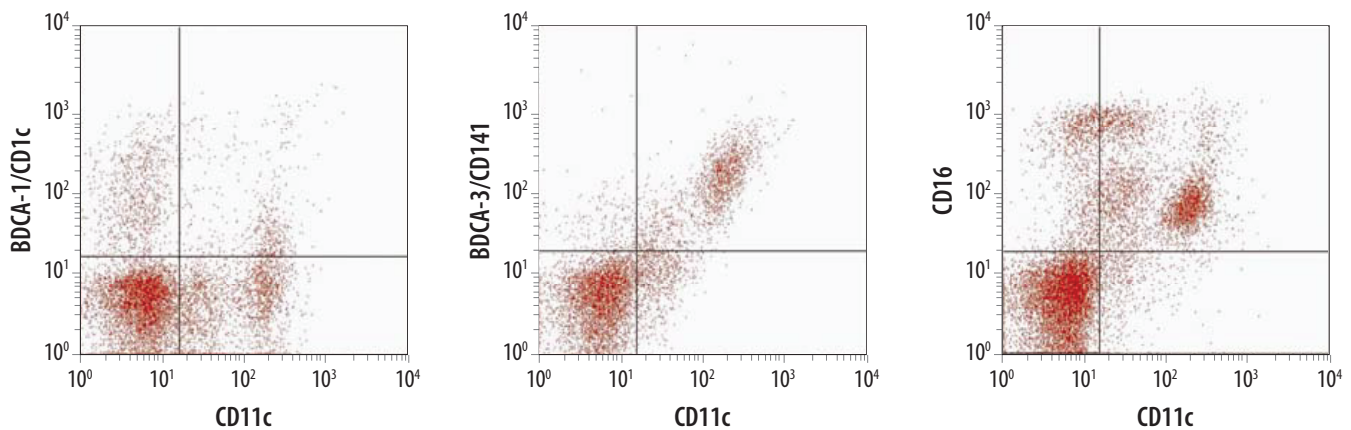
1. Piccioli, D. *et al.* (2009) *Blood* **109**:5371.
2. Osugi, Y. *et al.* (2002) *Blood* **100**:2858.
3. MacDonald, K.P.A. *et al.* (2002) *Blood*. **100**:4512.
4. Dzionek, A. *et al.* (2000) *J. Immunol.* **165**:6037.
5. Bagwell, B. and E.G. Adams (1993) *Ann. N.Y. Acad. Sci.* **677**:167.

## STAINING PROTOCOL

1. Cell samples should be washed with 2 mL of Staining Buffer, spinning the tube at 300 x g for 5 minutes.
2. Washed cells should be counted and then Fc receptor blocking reagents may be added. If using excess pre-immune IgG to block Fc receptor, use 1 µg of IgG per  $1 \times 10^5$  cells to be stained. The excess IgG does not need to be washed from the cells following the incubation period and can be carried into the staining reaction.
3. Transfer a small volume (about 100 µL) of the Fc receptor-blocked cells (about  $1 \times 10^6$  cells) into a 5 mL Flow Cytometry tube.
4. Add 10 µL of each antibody or each corresponding isotype control antibody to the cells.
5. Incubate the mixture for 30-45 minutes at room temperature **in the dark**.
6. Following the incubation, remove any excess antibody by washing the cells with 2 mL of Staining Buffer. The final cell pellet is resuspended in 200-400 µL of Staining Buffer for flow cytometric analysis.

**Note:** Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel (5).

## DATA EXAMPLES



**Figure 1:** Dot plots show PBMCs stained simultaneously with the indicated antibodies as described in the procedure. BDCA-1<sup>+</sup>/CD11c<sup>+</sup>, BDCA-3<sup>+</sup>/CD11c<sup>+</sup>, and CD16<sup>+</sup>/CD11c<sup>+</sup> are the main myeloid dendritic cell populations present in human blood. Quadrants were set based on isotype controls.