Human Myeloid Dendritic Cell
Multi-Color Flow Cytometry Kit

Catalog Number: FMC016

Size: 25 Tests

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.** Use within 6 months of receipt.

PART	PART #	DESCRIPTION
Positive Markers	967212	250 µL of BDCA-3/CD141-PE Mouse IgG_1 (Clone 501733)
	967213	250 µL of BDCA-1/CD1c-APC Goat IgG
	967214	250 µL of CD11c-CFS Mouse IgG_1 (Clone ICRF 3.9)
	967215	250 µL of CD16/Fcγ RIII-PerCP Mouse IgG_{2A} (Clone 245536)
Isotype Controls	965666	250 µL of Mouse IgG_1-PE
	967140	250 µL of Goat IgG-APC
	965668	250 µL of Mouse IgG_1-CFS
	967223	250 µL of Mouse IgG_{2A}-PerCP
Staining Buffer	895027	100 mL of 1X Staining Buffer

PRECAUTION

The Staining Buffers in this kit contain 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 x 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 x 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

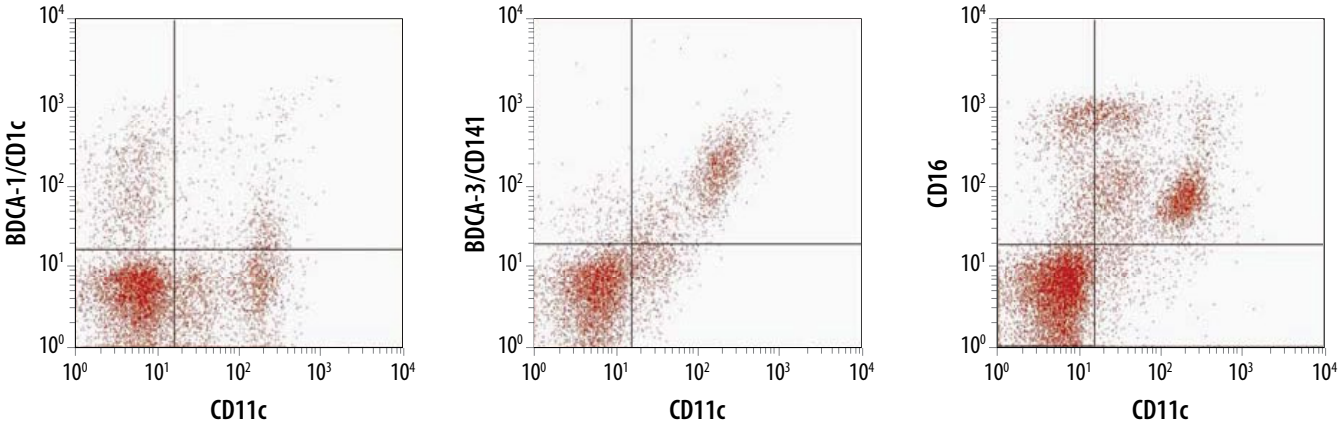

Figure 1: Dot plots show PBMCs stained simultaneously with the indicated antibodies as described in the procedure. BDCA-1$^+$/CD11c$^+$, BDCA-3$^+$/CD11c$^+$, and CD16$^+$/CD11c$^+$ are the main myeloid dendritic cell populations present in human blood. Quadrants were set based on isotype controls.