R&D SYSTEMS
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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.** Refer to the kit label for date of expiration.

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 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.

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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

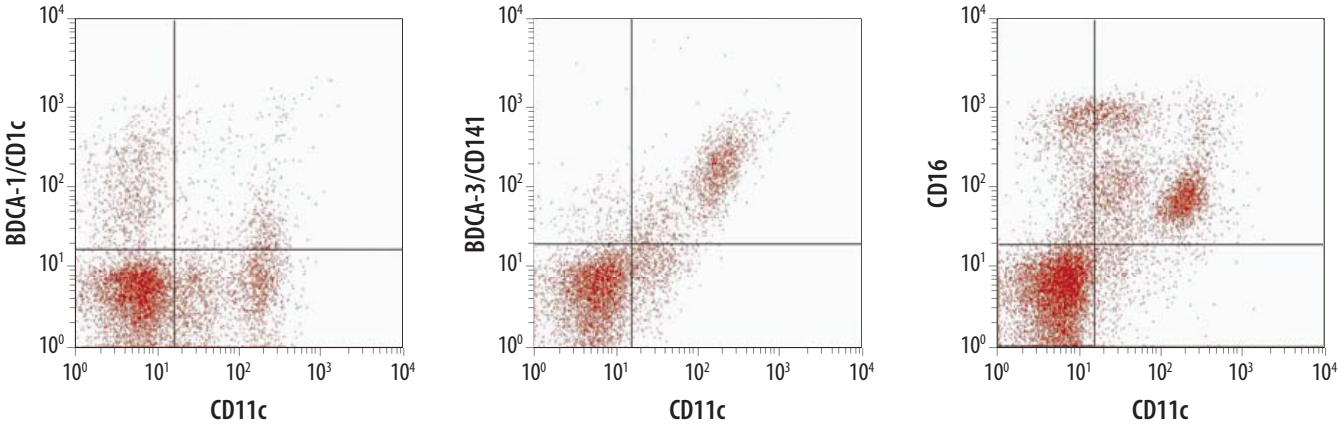

Figure 1: Dot plots show PBMCs stained simultaneously with the indicated antibodies as described in the procedure. BDCA-1$^+$/CD11c$^+$, BDCA-3$^+$/CD11c$^+$, and CD16$^+$/CD11c$^+$ are the main myeloid dendritic cell populations present in human blood. Quadrants were set based on isotype controls.