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 single-step staining of human myeloid dendritic cells (mDCs) (1 - 4):

- BDCA1/CD1c-APC (goat IgG)
- BDCA3/CD141-PE (Clone 501733; mouse IgG_1)
- CD16-PerCP (Clone 245536; mouse IgG_{2A})
- CD11c-CFS (Clone ICRF 3.9; mouse IgG_1)

This kit also contains Staining Buffer (100 mL).

Intended Use

This product is designed for the flow cytometric analysis of mDCs using four fluorochrome-conjugated antibodies.

Storage

Store at 2 - 8° C in the dark. Use within 6 months of receipt.

Precaution

The Staining Buffer contains 0.1% sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

Surface 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 2 - 8° C 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).*

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Typical Data

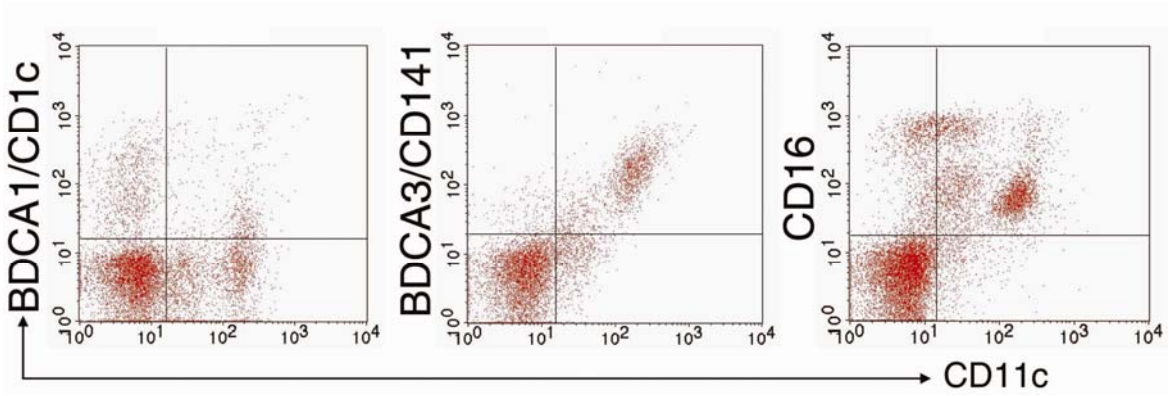

Figure 1: Dot plots show PBMCs stained simultaneously with the indicated antibodies as described in the procedure. BDCA1$^+$/CD11c$^+$, BDCA3$^+$/CD11c$^+$, and CD16$^+$/CD11c$^+$ are the main myeloid dendritic cell populations present in human blood. Quadrants were set based on isotype controls.

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.