Human Plasmacytoid Dendritic Cell Multi-Color Flow Cytometry Kit

Catalog Number: FMC017
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 plasmacytoid dendritic cells (pDCs) (1 - 4):

- 250 µL of DLEC/CLEC4C/BDCA-2-PE Goat IgG (Part 967216)
- 250 µL of CD45-APC Mouse IgG_1; Clone 2D1 (Part 967217)
- 250 µL of CD123/IL-3R-PerCP Mouse IgG_1; Clone 32703 (Part 967218)
- 250 µL of BDCA-4-CFS Mouse IgG_{2A}; Clone 446921 (Part 967219)

This kit also contains 100 mL of Staining Buffer (Part 895027).

Intended Use

This product is designed for the flow cytometric analysis of pDCs 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

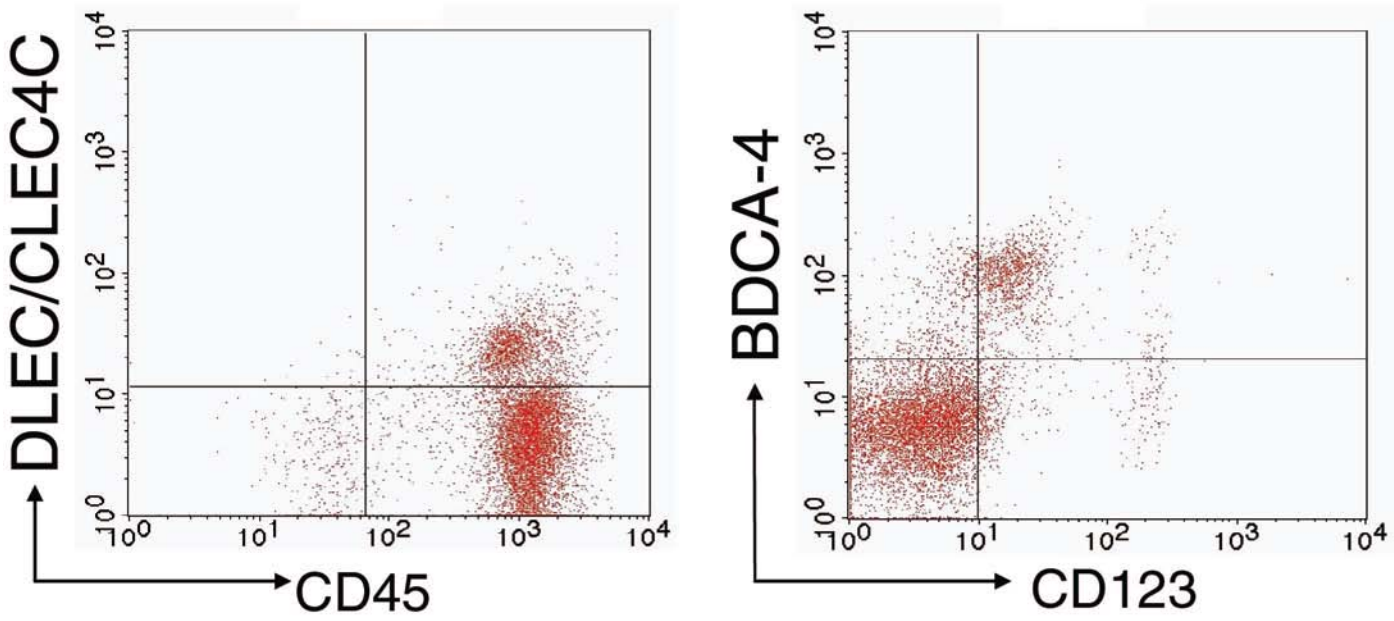

Figure 1: Dot plots show staining with the indicated antibodies of plasmacytoid dendritic cells enriched from PBMCs with the MagCellect Human Blood Dendritic Cell Isolation Kit (R&D Systems, Catalog # MAGH110), as described in the procedure. Quadrants were set based on isotype controls.

References

1. Dzionek, A. *et al*. (2001) J. Exp. Med. **194**:1823.
2. Marafioti, T. *et al*. (2008) Blood **111**:3778.
3. Lande, R. *et al*. (2007) Nature **449**:564.
4. Farkas, L. *et al*. (2001) Am. J. Pathol. **159**:237.
5. Bagwell, B. and E.G. Adams (1993) Ann. N.Y. Acad. Sci. **677**:167.