



Human Multipotent Mesenchymal Stromal Cell Multi-Color Flow Cytometry Kit

Catalog Number: FMC002

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 multipotent mesenchymal stromal cells (hMSCs) (1 - 4):

Positive Markers

- CD105-PerCP (Clone 166707; mouse IgG₁)
- CD146-CFS (Clone 128018; mouse IgG₁)
- CD90-APC (Clone Thy-1A1; mouse IgG_{2A})

Negative Marker

- CD45-PE (Clone 2D1; mouse IgG₁)

This kit also contains Staining Buffer (100 mL).

Intended Use

This product is designed for the flow cytometric analysis of hMSCs 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 10⁵ 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 10⁶ 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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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

R&D Systems, Inc.

1-800-343-7475

Typical Data

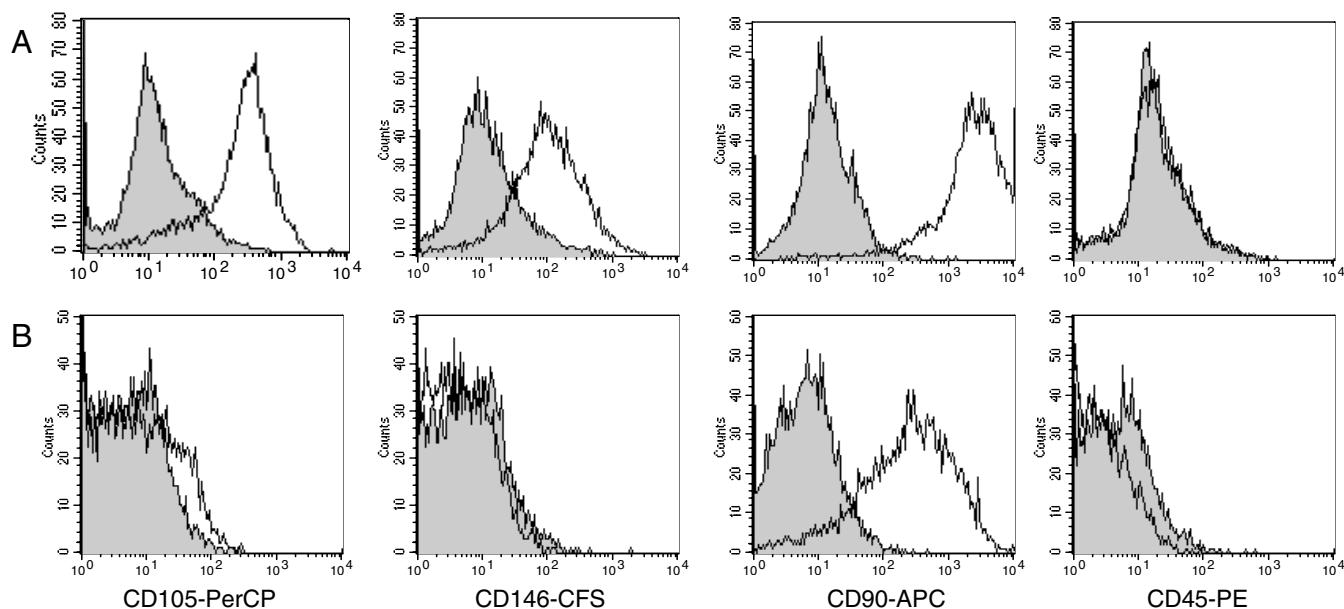


Figure 1: Undifferentiated (A) and osteocyte-differentiated (B) hMSCs were stained with the indicated antibodies (open histograms) or the corresponding isotype control (filled histograms), as described in the procedure. Osteocyte-differentiated (21 days) cells show a characteristic reduction in CD146, CD105, and CD90 staining. CD45 is a negative control for both cell types.

References

1. Chamberlain, G. *et al.* (2007) *Stem Cells* **25**:2739.
2. Abdallah, B.M. and M. Kassem (2008) *Gene Therapy* **15**:109.
3. Spitkovsky, D. and J. Hescheler (2008) *Min. Inv. Ther.* **17**:79.
4. Delorme, B. *et al.* (2008) *Blood* **111**:2631.
5. Bagwell, B. and E.G. Adams (1993) *Ann. N.Y. Acad. Sci.* **677**:167.