R&D SYSTEMS
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Human Mesenchymal Stem Cell
Multi-Color Flow Cytometry Kit

Catalog Number: FMC002

Size: 25 Tests

PRODUCT DESCRIPTION

This kit contains four conjugated antibodies and four corresponding isotype controls that can be used for single-step staining of human mesenchymal stem/stromal cells (MSCs) (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	965665	250 µL of CD105/Endoglin-PerCP Mouse IgG_1; Clone 166707
	965664	250 µL of CD146/MCAM-CFS Mouse IgG_1; Clone 128018
	965663	250 µL of CD90/Thy1-APC Mouse IgG_{2A}; Clone Thy-1A1
Negative Marker	965662	250 µL of CD45-PE Mouse IgG_1; Clone 2D1
Isotype Controls	965666	250 µL of Mouse IgG_1-PE Isotype Control
	965667	250 µL of Mouse IgG_{2A}-APC Isotype Control
	965668	250 µL of Mouse IgG_1-CFS Isotype Control
	965669	250 µL of Mouse IgG_1-PerCP Isotype Control
Staining Buffer	895027	100 mL of 1X Staining Buffer

INTENDED USE

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

PRECAUTION

The Staining Buffer contains 0.09% 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 desired. 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 of cells (1 x 10^6 cells in 100 µL is recommended) into a 5 mL Flow Cytometry tube.
4. Add 10 µL of each antibody, or add 10 µL of 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).*

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DATA EXAMPLES

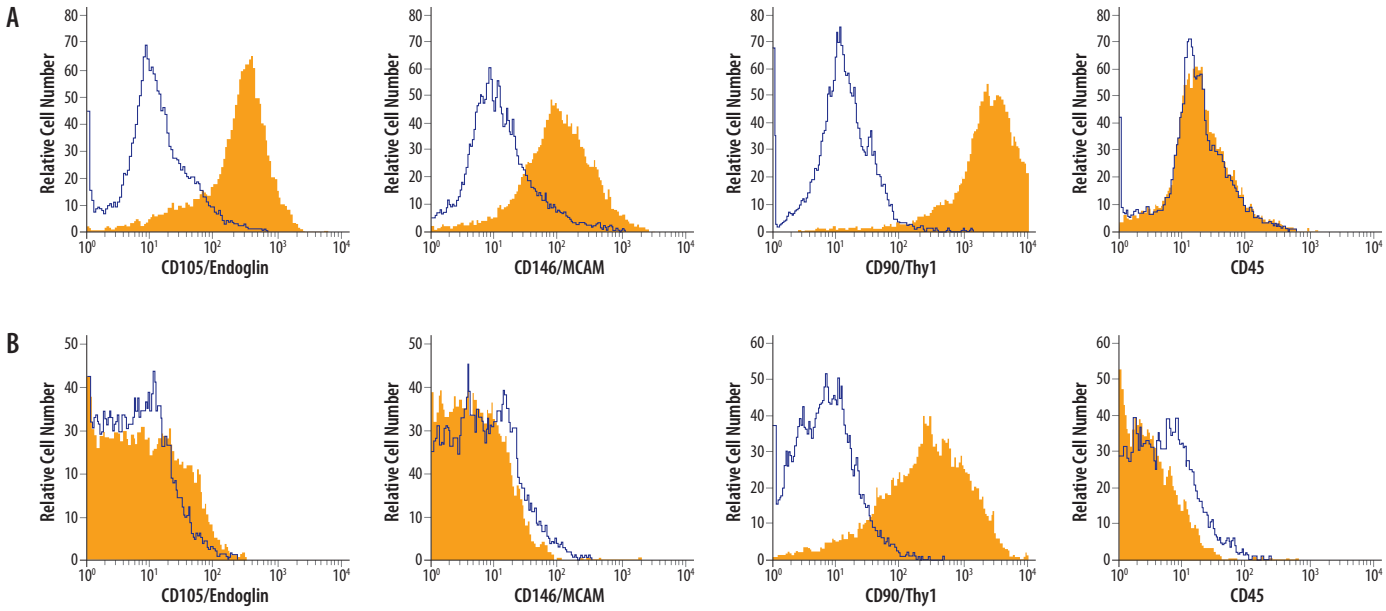

Figure 1: Verification of Human Mesenchymal Stem/Stromal Cell Identity by Analysis of MSC Marker Expression. Undifferentiated **(A)** and osteocyte-differentiated **(B)** human MSCs were stained with the indicated antibodies (filled histograms) or the corresponding isotype control (open histograms), as described in the procedure. Multi-color flow cytometry simultaneously detected cells that were positive for MSC markers (CD105/Endoglin, CD146/MCAM, and CD90/Thy1). Following 21 days of osteocyte-differentiation, the cells showed a characteristic reduction in CD105/Endoglin, CD146/MCAM, and CD90/Thy1 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.