Mouse Mesenchymal Stem Cell
Multi-Color Flow Cytometry Kit

Catalog Number: FMC003
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 mouse mesenchymal stem/stromal cells (MSCs) (1-4).

MATERIALS PROVIDED & STORAGE

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

PART	PART #	DESCRIPTION
Positive Markers	967207	250 µL of CD105/Endoglin-CFS Rat IgG_{2A}; Clone 209701
	967205	250 µL of CD29/Integrin β1-PE Rat IgG_{2A}; Clone 265917
	967206	250 µL of Sca-1-APC Rat IgG_{2A}; Clone 177228
Negative Marker	967208	250 µL of CD45-PerCP Rat IgG_{2B}; Clone 30-F11
Isotype Controls	967209	250 µL of Rat IgG_{2A}-PE Isotype Control
	965948	250 µL of Rat IgG_{2A}-APC Isotype Control
	965715	250 µL of Rat IgG_{2A}-CFS Isotype Control
	967114	250 µL of Rat IgG_{2B}-PerCP Isotype Control
Staining Buffer	895027	100 mL of 1X Staining Buffer

INTENDED USE

This product is designed for the flow cytometric analysis of mouse 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).*

DATA EXAMPLES

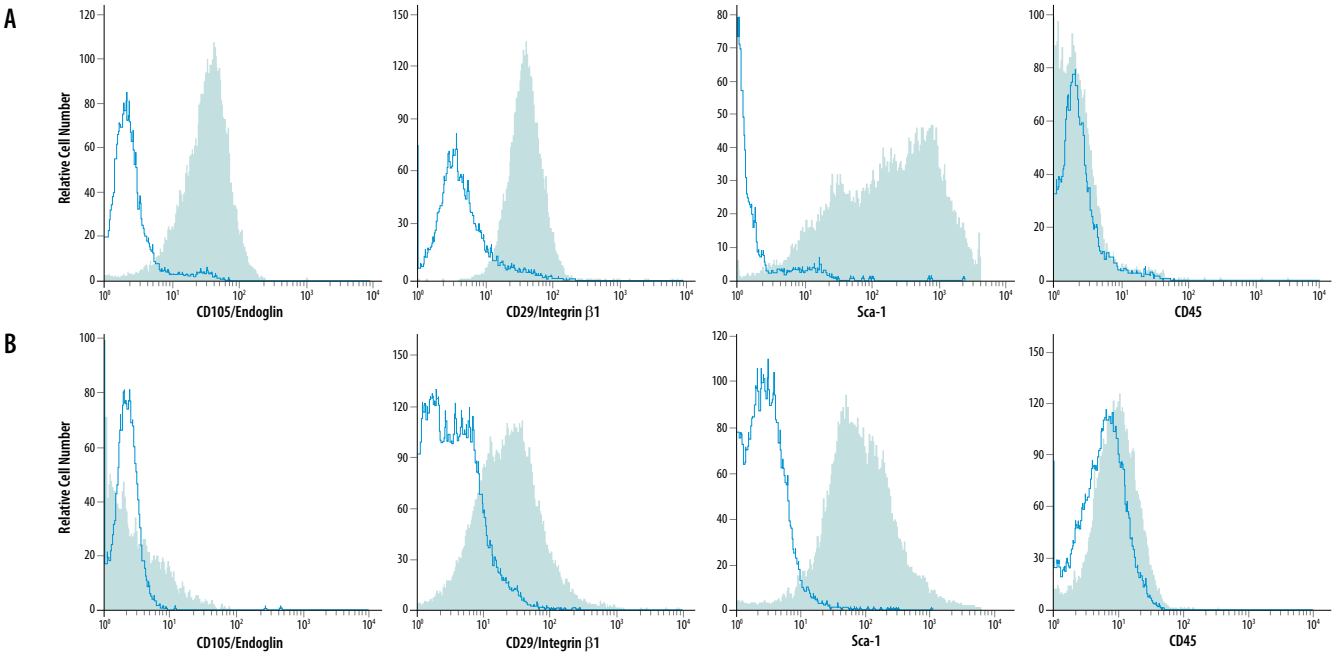

Figure 1: Verification of Mouse Mesenchymal Stem/Stromal Cell Identity by Analysis of MSC Marker Expression. Undifferentiated **(A)** and osteocyte-differentiated **(B)** mouse MSCs were stained with the indicated antibodies (filled histograms) or the corresponding isotype control (open histograms), as described in the procedure. Mouse MSCs demonstrate the characteristic expression of CD105/Endoglin, CD29/Integrin β1, and Sca-1. Following 20 days of osteocyte-differention, cells show a characteristic reduction in CD105, CD29, and Sca-1 staining. CD45 is a negative control for both cell types.

REFERENCES

1. Bernardo, M.E. *et al.* (2009) Ann. N.Y. Acad. Sci. **1176**:101.
2. Short, B.J. *et al.* (2009) Meth. Mol. Biol. **482**:259.
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4. Rombouts, W.J. and R.E. Ploemacher (2003) Leukemia **17**:160.
5. Bagwell, B. and E.G. Adams (1993) Ann. N.Y. Acad. Sci. **677**:167.