Mouse Mesenchymal Stem Cell Marker Antibody Panel

Catalog Number SC018

Reagents for the identification of mouse mesenchymal stem/stromal cells.

This package insert must be read in its entirety before using this product.

For laboratory research use only. Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

TABLE OF CONTENTS

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INTRODUCTION

Originally identified in the non-hematopoietic compartment of the bone marrow, human mesenchymal stem cells exhibit multi-lineage differentiation capacity, can be greatly expanded *in vitro*, and adhere to plastic (1-4; see Figure 1). Although originally referred to as mesenchymal stem cells, the cells are now also referred to as mesenchymal stromal cells. This change in nomenclature was based on the absence of direct evidence for MSC "stemness" *in vivo* and the fact that not all cells within the population display stem cell characteristics. The International Society for Cellular Therapy (ISCT) proposed the change in nomenclature and further suggested three minimal criteria for defining human mesenchymal stromal cells including 1) MSCs adhere to plastic, 2) MSCs can differentiate into osteoblasts, adipocytes, and chondrocytes, and 3) MSCs demonstrate positive expression of CD105, CD73, and CD90/Thy1, and negative expression of CD45, CD34, HLA-DR, CD14 or CD11b, CD79α, and CD19 (5).

Although similar guidelines have not been established for non-human MSCs, it is generally accepted that the minimal criteria for the definition of human MSCs are applicable to other species with slight differences in surface markers. Using markers to define mouse MSCs is complicated by the finding that the mouse strain can affect marker expression. Sca-1 and CD90 are two markers that may differ in expression depending on strain (6-8). Despite these strain-dependent differences, it is generally accepted that mouse MSCs positively express CD106, CD105, CD73, CD29, CD44, and Sca-1 (6, 9-11). These positive cell markers together with the absence of hematopoietic and endothelial markers Ter-119, CD45, CD11b, and CD31 are now routinely used to characterize mouse MSCs (6, 9, 12). Because there are no markers that are exclusive to mouse MSCs, a panel of acceptable surface markers is commonly used for the characterization of both primary and cultured MSCs.

Researchers use different techniques to isolate, culture, and differentiate mesenchymal stem/stromal cells (MSCs). Variations in experimental approach as well as differences in the MSC starting population may account for experimental variability and explain some of the contradictory data that have been published in the stem cell field. One way to minimize experimental variation is to clearly define the starting cell population by using a functional or phenotypic assay. R&D Systems offers the Mouse Mesenchymal Stem Cell Marker Antibody Panel, which uses expression of multiple established markers to assess MSC identity.

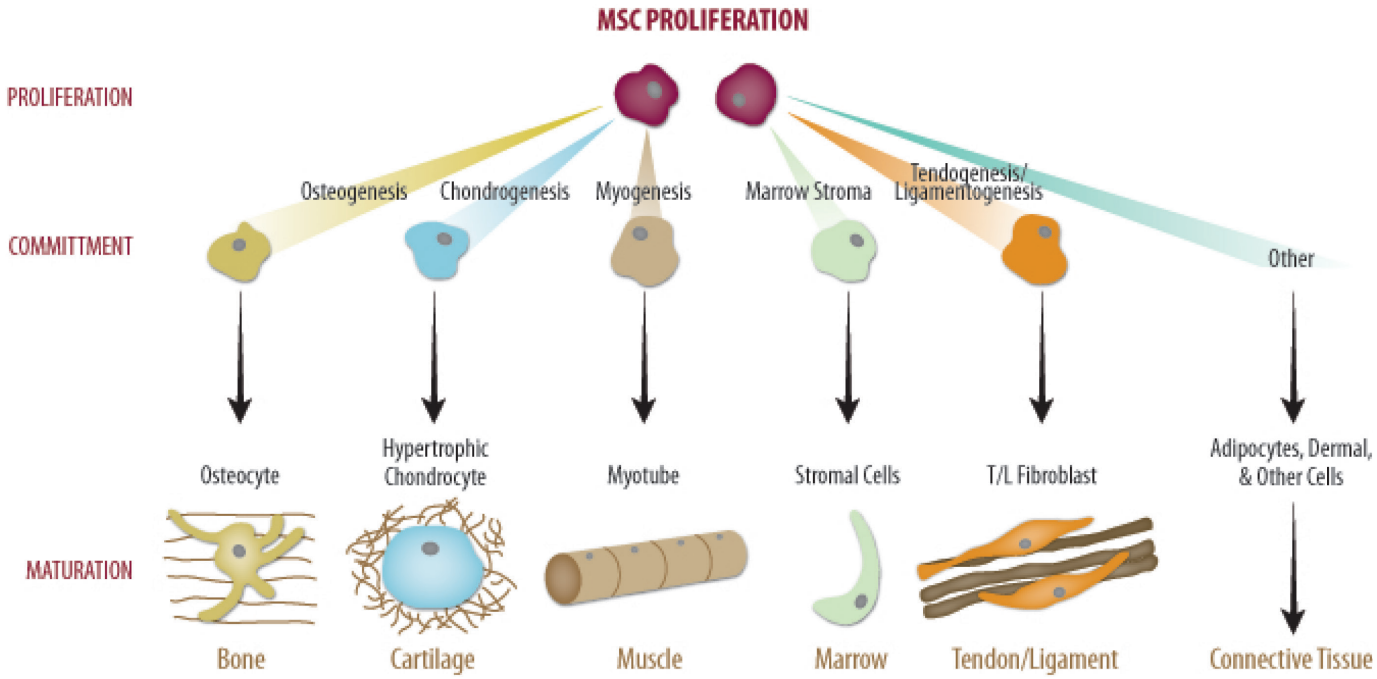

Figure 1: The differentiation potential of MSC.

DESIGN OF THE PANEL

The Mouse Mesenchymal Stem Cell Marker Antibody Panel is designed for the identification and characterization of cultured or freshly isolated mouse multipotent mesenchymal stem/stromal cells. The panel contains a group of antibodies for the positive (anti-Sca-1, anti-CD106, anti-CD105, anti-CD73, anti-CD29, and anti-CD44) and negative (anti-CD11b and anti-CD45) identification of mouse MSC.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses have not been established.

PRECAUTION

The acute and chronic effects of over-exposure to reagents in this kit are unknown. Safe laboratory procedures should be followed and protective clothing should be worn when handling kit reagents.

MATERIALS PROVIDED

Note: *Optimal dilutions should be determined by each laboratory for each application.*

PART	PART #	DESCRIPTION
Anti-Sca-1 (Clone 177228; isotype rat IgG_{2A})	965955	25 µg of lyophilized rat anti-mouse Sca-1 monoclonal antibody.
Anti-CD106 (Clone 112734; isotype rat IgG_{2A})	965956	25 µg of lyophilized rat anti-mouse CD106 monoclonal antibody.
Anti-CD105 (Clone 209701; isotype rat IgG_{2A})	965957	25 µg of lyophilized rat anti-mouse CD105 monoclonal antibody.
Anti-CD73 (Clone 496406; isotype rat IgG_{2A})	965958	25 µg of lyophilized rat anti-mouse CD73 monoclonal antibody.
Anti-CD29 (Clone 265917; isotype rat IgG_{2A})	965959	25 µg of lyophilized rat anti-mouse CD29 monoclonal antibody.
Anti-CD11b (Clone M1/70; isotype rat IgG_{2B})	965961	25 µg of lyophilized rat anti-mouse CD11b monoclonal antibody.
Anti-CD45 (Clone 30-F11; isotype rat IgG_{2B})	965962	25 µg of lyophilized rat anti-mouse CD45 monoclonal antibody.
Anti-CD44	967443	25 µg of lyophilized sheep anti-mouse/rat CD44 polyclonal antibody.

STORAGE

Unopened Kit	Store at 2-8 ° C. Use within 1 year of receipt.
Opened/Reconstituted Reagents	May be stored for up to 1 month at 2-8 °C.* Aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 6 months.* Avoid repeated freeze-thaw cycles.

*Provided this is within 1 year from receipt.

OTHER SUPPLIES REQUIRED

- Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001)
- Isotype controls (R&D Systems, Catalog # MAB006, MAB0061, and 5-001-A)
- Rat secondary developing reagents (R&D Systems, Catalog # F0113, F0104B, F0105B, and F0115)
- Sheep secondary developing reagents (R&D Systems, Catalog # F0125, F0126, F0127, and F0128)
- Sterile PBS
- Benchtop centrifuge

REAGENT PREPARATION

Reconstitute each vial with 250 µL of sterile PBS. This provides reagents sufficient for processing 25 samples.

PROCEDURE

Use serological pipettes to transfer and remove solutions.

Surface Marker Analysis by Flow Cytometry

1. Resuspend the cells in Flow Cytometry Staining Buffer at a concentration of 1×10^6 cells/mL.
2. For each marker, transfer 90 µL of the cell suspension into a separate 5 mL tube. Add 10 µL of antibody.
3. Incubate for 30 minutes at room temperature.
4. Following incubation, wash the sample twice in 2 mL of Flow Cytometry Staining Buffer.
5. Resuspend the cells in 100 µL of Flow Cytometry Staining Buffer, and add the appropriate secondary developing reagent such as anti-rat IgG or anti-sheep IgG conjugated to a fluorochrome according to the manufacturer's instructions.
6. Incubate for 30 minutes at room temperature **in the dark.**
7. Following incubation, wash the sample twice in 2 mL of Flow Cytometry Staining Buffer.
8. Resuspend the cells in 200 µL of Flow Cytometry Staining Buffer for flow cytometric analysis.

   Note: *As a control for analysis, cells in a separate tube should be treated with isotype control.*

TYPICAL DATA

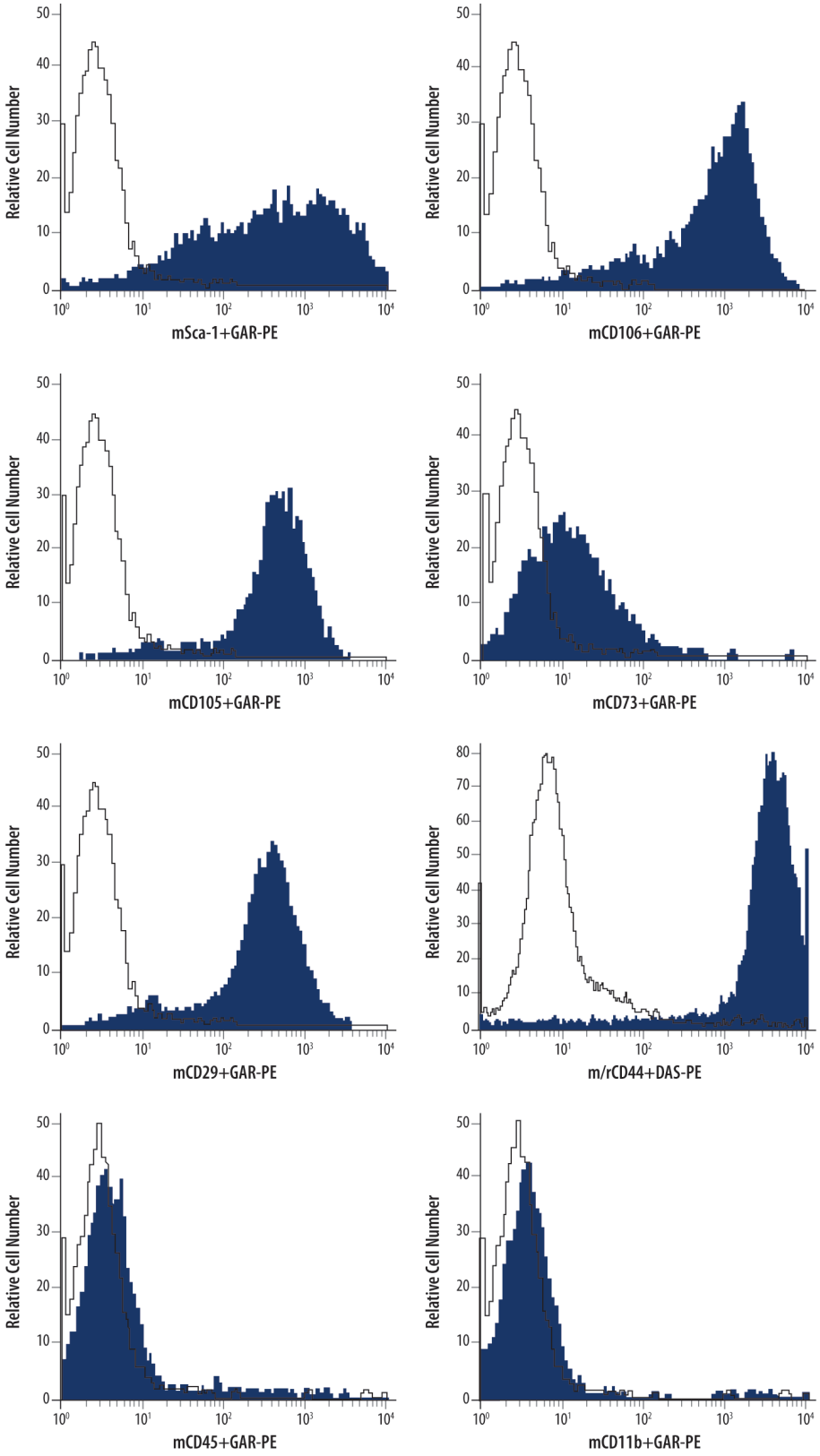

Figure 2: Flow cytometric analysis of an immortalized mouse MSC clone (13) using the antibodies indicated (filled histograms) with secondary developing reagents conjugated to phycoerythrin. The cells were stained according to the procedure on page 4. The respective isotype controls are also shown (empty histograms).

RELATED REAGENTS

Product Description	R&D Systems Catalog Number
Anti-mouse Sca-1 Monoclonal Antibody (Clone 177228)	MAB1226
Anti-mouse CD106 Monoclonal Antibody (Clone 112734)	MAB6432
Anti-mouse CD105 Monoclonal Antibody (Clone 209701)	MAB1320
Anti-mouse CD73 Monoclonal Antibody (Clone 496406)	MAB4488
Anti-mouse CD29 Monoclonal Antibody (Clone 265917)	MAB2405
Anti-mouse CD11b Monoclonal Antibody (Clone M1/70)	MAB1124
Anti-mouse CD45 Monoclonal Antibody (Clone 30-F11)	MAB114
Anti-mouse/rat CD44 Polyclonal Antibody	AF6127
Goat $F(ab)_2$ Anti-rat IgG (H+L) Allophycocyanin	F0113
Goat $F(ab)_2$ Anti-rat IgG (H+L) Phycoerythrin	F0105B
Goat $F(ab)_2$ Anti-rat IgG (H+L) Fluorescein	F0104B
Goat $F(ab)_2$ Anti-rat IgG (H+L) PerCP	F0115
Rat IgG_{2A} Isotype Control (Clone 54447)	MAB006
Rat IgG_{2B} Isotype Control (Clone 141945)	MAB0061
Normal Sheep IgG Control	5-001-A
Mouse Mesenchymal Stem Cell Functional Identification Kit	SC010

REFERENCES

1. Friedenstein, A.J. *et al*. (1976) Exp. Hemat. **4**:267.
2. Keating, A. (2012) Cell Stem Cell **10**:709.
3. Pittenger, M.F. *et al*. (1999) Science **284**:143.
4. Prockop, D.J. *et al*. (1997) Science **276**:71.
5. Dominici, M. *et al*. (2006) Cytotherapy **8**:315.
6. Peister, A. *et al*. (2004) Blood **103**:662.
7. Phinney, D.G. *et al*. (1999) J. Cell Biochem. **72**:570.
8. Rege, T.A. & J.S. Hagood (2006) FASEB J. **20**:1045.
9. Baddoo, M. *et al*. (2003) J. Cell Biochem. **89**:1235.
10. Meirelles, L.S. & N.B. Nardi (2003) Br. J. Haematol. **123**:702.
11. Sun, S. *et al*. (2003) Stem Cells **21**:527.
12. Tropel, P. *et al*. (2004) Exp. Cell Res. **295**:395.
13. Dennis, J.E. & A.I. Caplan (1996) J. Cell. Physiol. **167**:523.