

Human Multipotent Mesenchymal Stromal Cell Marker Antibody Panel

Catalog Number SC017

**Reagents for the identification of human multipotent
mesenchymal 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.**

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INTRODUCTION

Multipotent mesenchymal stromal cells (MSC) are the cells originally identified in the non-hematopoietic compartment of bone marrow, which exhibit multi-lineage differentiation capacity and can be greatly expanded *in vitro* (1 - 3; see Figure 1). These cells are also known as marrow stromal cells or mesenchymal stem cells (4). However, due to the lack of direct evidence proving the “stemness” of these cells in many publications, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) has issued a position statement and designated the name “multipotent mesenchymal stromal cells” (to replace the misnomer mesenchymal stem cells), thus avoiding the scientific implication of “stem cells” associated with MSC (5).

MSCs are plastic-adherent cells that exist in low frequency (estimated to be 0.001 - 0.01%) in bone marrow. They can be isolated from trabecular bone, adipose tissue, skeletal muscle, deciduous teeth, blood, cord blood, and other adult tissues. In recent years, the scientific interest in MSC soared as their versatile roles were uncovered. In addition to the multipotent differentiation potential and a supportive role in hematopoiesis, MSC demonstrate remarkable trophic effects and immunomodulatory potential (6 - 9). As a result of the diverse cell sources, isolation methods, and culturing protocols described by different laboratories, the Mesenchymal and Tissue Stem Cell Committee has issued a guideline for the characterization of MSC (10). Three minimum criteria are plastic-adherent, specific surface antigen expression (see Table 1), and *in vitro* differentiation potential into osteoblasts, adipocytes, and chondroblasts. In spite of the establishment of a guideline, characterization of MSC may still vary from laboratory to laboratory. One of the reasons is the unavailability of MSC-specific surface marker(s). Several monoclonal antibodies had been generated for use in characterization but were later found to be non-specific. These antibodies included Stro-1, SB-10 (specific for CD166), SH-2 (specific for endoglin/CD105), as well as SH-3 and SH-4 (specific for CD73) (11 - 13). Additional positive markers have been identified on MSC and can be found on other cell types including CD146, CD90, CD44, and CD29, among others (14). These positive cell surface markers, together with the absence of hematopoietic markers CD34, CD45, CD14, and CD19, are now routinely used in MSC characterization (15). Despite the lack of a specific marker, the use of flow cytometry to characterize both primary and cultured MSC remains a common practice given that a panel of approved surface markers is used.

Table 1: Surface markers expression on MSC.

Positive Marker ($\geq 95\%$)	Negative Marker ($\leq 2\%$)
CD105	CD45
CD73	CD34
CD90	CD14 or CD11b
	CD79 α or CD19
	HLA-DR

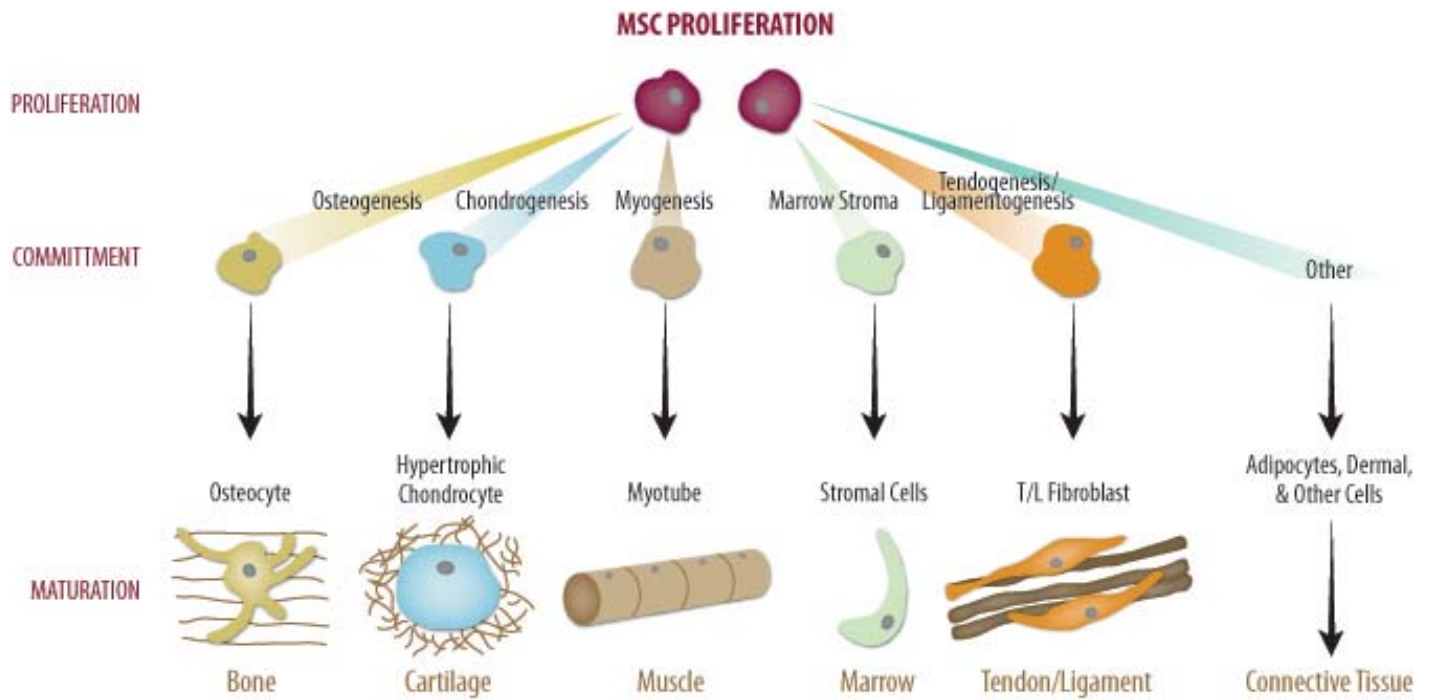


Figure 1: The differentiation potential of MSC.

DESIGN OF THE PANEL

The Human Multipotent Mesenchymal Stromal Cell Marker Antibody Panel is designed for the identification and characterization of cultured or freshly isolated human multipotent mesenchymal stromal cells. The panel contains a group of antibodies for the positive (anti-Stro-1, anti-CD90, anti-CD106, anti-CD105, anti-CD146, anti-CD166, and anti-CD44) and negative (anti-CD19 and anti-CD45) identification of 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

Anti-Stro-1 (clone Stro-1; isotype mouse IgM λ ; Part # 965608) - 25 μ g of lyophilized mouse anti-human Stro-1 monoclonal antibody.

Anti-CD90 (clone Thy-1A1; isotype mouse IgG_{2A}; Part # 965609) - 25 μ g of lyophilized mouse anti-human CD90 monoclonal antibody.

Anti-CD106 (clone BBIG-V1 [4B2]; isotype mouse IgG₁; Part # 965610) - 25 μ g of lyophilized mouse anti-human CD106 monoclonal antibody.

Anti-CD105 (clone 166707; isotype mouse IgG₁; Part # 965611) - 25 μ g of lyophilized mouse anti-human CD105 monoclonal antibody.

Anti-CD146 (clone 128018; isotype mouse IgG₁; Part # 965612) - 25 μ g of lyophilized mouse anti-human CD146 monoclonal antibody.

Anti-CD166 (clone 105902; isotype mouse IgG₁; Part # 965613) - 25 μ g of lyophilized mouse anti-human CD166 monoclonal antibody.

Anti-CD44 (clone 2C5; isotype mouse IgG_{2A}; Part # 965614) - 25 μ g of lyophilized mouse anti-human CD44 monoclonal antibody.

Anti-CD19 (clone 4G7-2E3; isotype mouse IgG₁; Part # 965615) - 25 μ g of lyophilized mouse anti-human CD19 monoclonal antibody.

Anti-CD45 (clone 2D1; isotype mouse IgG₁; Part # 965616) - 25 μ g of lyophilized mouse anti-human CD45 monoclonal 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 \leq -20° C in a manual defrost freezer for up to 6 months. Avoid repeated freeze-thaw cycles.

OTHER SUPPLIES REQUIRED

- Flow Cytometry Staining Buffer (R&D Systems, Catalog # FC001)
- Isotype controls (R&D Systems, Catalog # MAB002 and MAB003; Caltag[®], Catalog # MGM00)
- Secondary developing reagents (R&D Systems, Catalog # F0101B, F0102B, F0103B, F0114, F0116, F0117, F0118, and F0119)
- Sterile PBS
- Benchtop centrifuge
- 2 - 8° C refrigerator

REAGENT PREPARATION

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

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

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 2 - 8° C.
4. Following incubation, wash the sample twice in 2 mL of Flow Cytometry Staining Buffer.
5. Resuspend the cells in 200 µL of Flow Cytometry Staining Buffer and add a secondary developing reagent such as anti-mouse IgG or anti-mouse IgM conjugated to a fluorochrome according to the manufacturer's instructions.
6. Incubate for 30 minutes at 2 - 8° C **in the dark**.
7. Following incubation, wash the sample twice in 2 mL of Flow Cytometry Staining Buffer.
8. Resuspend the cells in 400 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.*

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TYPICAL DATA

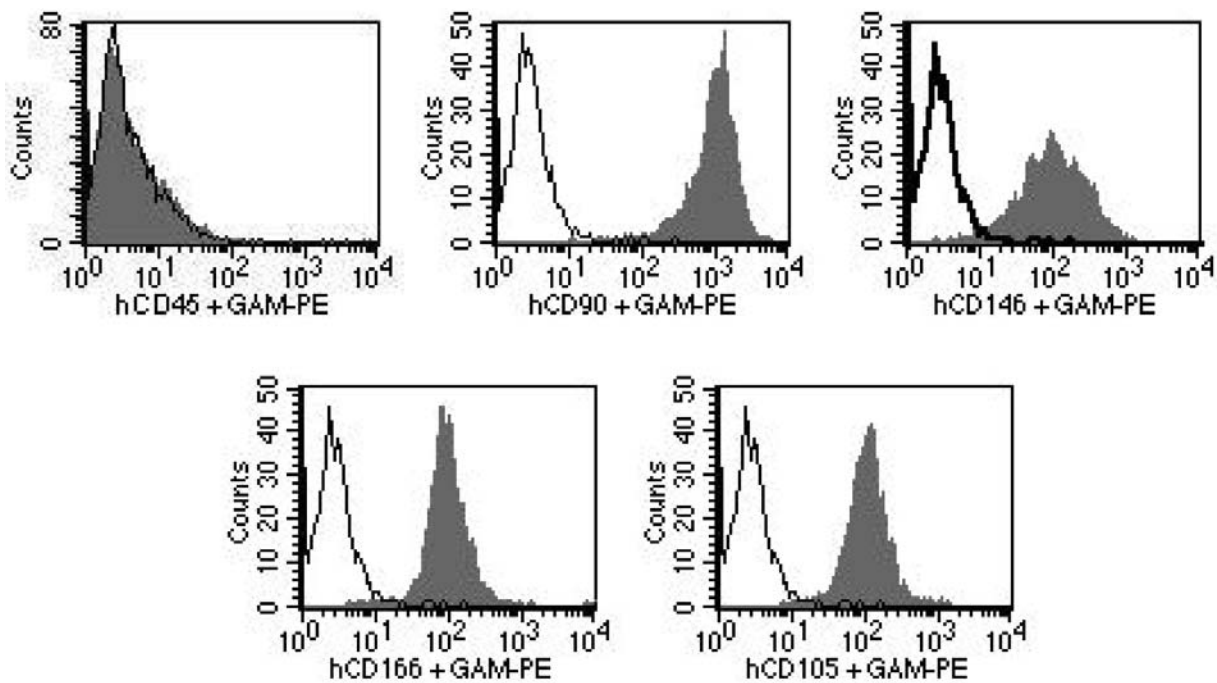


Figure 1: Flow cytometric analysis of human MSC using the monoclonal antibodies indicated (filled histograms) with secondary developing reagent goat anti-mouse (GAM) conjugated to phycoerythrin. The cells were stained according to the instructions on page 5. The respective isotype controls are also shown (empty histograms).

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8. Di Nicola, M. *et al.* (2002) *Blood* **99**:3838.
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12. Bruder, S.P. *et al.* (1997) *Bone* **21**:225.
13. Haynesworth, S.E. *et al.* (1992) *Bone* **13**:69.
14. Kolf, C.M. *et al.* (2007) *Arthritis Res. Ther.* **9**:204.
15. Delorme, B. *et al.* (2008) *Blood* **111**:2631.

RELATED REAGENTS

Product Description	R&D System's Catalog Number
Anti-human Stro-1 Monoclonal Antibody (Clone Stro-1)	MAB1038
Anti-human CD90 Monoclonal Antibody (Clone Thy-1A1)	MAB2067
Anti-human CD106 Monoclonal Antibody (Clone BBIG-V1)	BBA5
Anti-human CD105 Monoclonal Antibody (Clone 166707)	MAB10971
Anti-human CD146 Monoclonal Antibody (Clone 128018)	MAB932
Anti-human CD166 Monoclonal Antibody (Clone 105902)	MAB6561
Anti-human CD44 Monoclonal Antibody (Clone 2C5)	BBA10
Anti-human CD19 Monoclonal Antibody (Clone 4G7-2E3)	MAB4867
Anti-human CD45 Monoclonal Antibody (Clone 2D1)	MAB1430
Goat F(ab') ₂ Anti-Mouse IgG (H+L) Allophycocyanin	F0101B
Goat F(ab') ₂ Anti-Mouse IgG (H+L) Phycoerythrin	F0102B
Goat F(ab') ₂ Anti-Mouse IgG (H+L) Fluorescein	F0103B
Goat F(ab') ₂ Anti-Mouse IgG (H+L) PerCP	F0114
Goat Anti-Mouse IgM Phycoerythrin	F0116
Goat Anti-Mouse IgM Allophycocyanin	F0117
Goat Anti-Mouse IgM Fluorescein	F0118
Goat Anti-Mouse IgM PerCP	F0119
Mouse IgG ₁ Isotype Control (Clone 11711)	MAB002
Mouse IgG _{2A} Isotype Control (Clone 20102)	MAB003
Human Mesenchymal Stem Cell Functional Identification Kit	SC006