

INTRODUCTION

Mesenchymal stem 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 mesenchymal or marrow stromal cells (4). 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, 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.

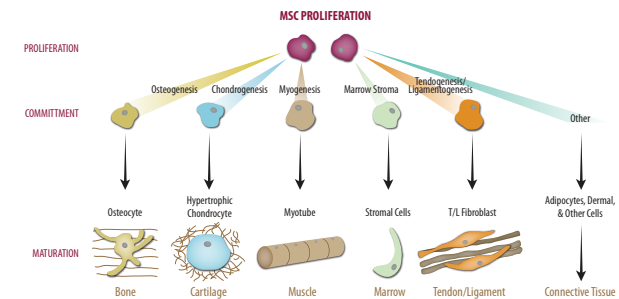


Figure 1: The differentiation potential of MSCs.

DATA EXAMPLES

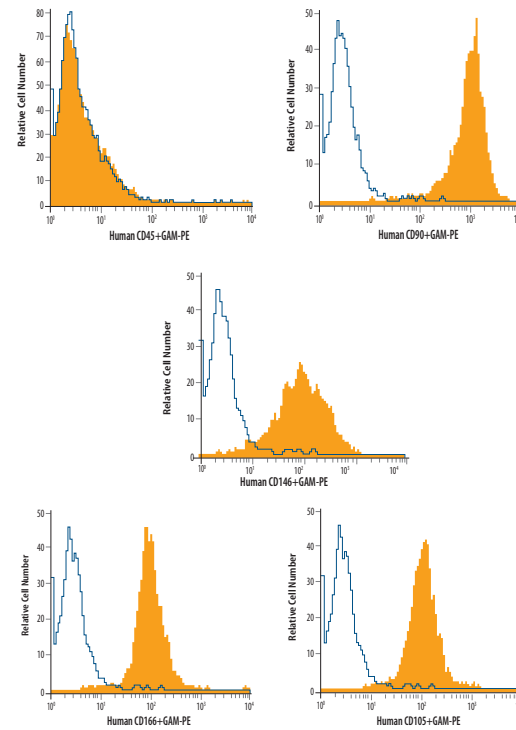


Figure 1: Flow cytometric analysis of human bone marrow-derived MSCs using the monoclonal antibodies indicated (filled histograms) with secondary developing reagent goat anti-mouse (GAM) conjugated to phycoerythrin (PE). The cells were stained according to the procedure provided in this insert. The respective isotype controls are also shown (empty histograms).

REFERENCES

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13. Haynesworth, S.E. *et al.* (1992) *Bone* **13**:69.
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NOTES

Human Mesenchymal Stem Cell Marker Antibody Panel

Catalog Number: SC017

PRINCIPLE OF THE ASSAY

The Human Mesenchymal Stem Cell Marker Antibody Panel is designed for the identification and characterization of cultured or freshly isolated human mesenchymal stem/stromal cells (MSCs). 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 MSCs.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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MATERIALS PROVIDED & STORAGE CONDITIONS

Store unopened kit at 2-8 °C for up to 1 year from date of receipt.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Anti-Stro-1 Purified Mouse Monoclonal IgM Clone: STRO-1	965608	25 µg of a mouse monoclonal antibody specific for human Stro-1; lyophilized.	Store at 2-8 °C for up to 1 month or aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 6 months*. Avoid repeated freeze-thaw cycles.
Anti-CD90 Purified Mouse Monoclonal IgG ₁ Clone: Thy-1A1	965609	25 µg of a mouse monoclonal antibody specific for human CD90; lyophilized.	
Anti-CD106 Purified Mouse Monoclonal IgG ₁ Clone: BBIG-V1 [4B2]	965610	25 µg of a mouse monoclonal antibody specific for human CD106; lyophilized.	
Anti-CD105 Purified Mouse Monoclonal IgG ₁ Clone: 166707	965611	25 µg of a mouse monoclonal antibody specific for human CD105; lyophilized.	
Anti-CD146 Purified Mouse Monoclonal IgG ₁ Clone: 128018	965612	25 µg of a mouse monoclonal antibody specific for human CD146; lyophilized.	
Anti-CD166 Purified Mouse Monoclonal IgG ₁ Clone: 105902	965613	25 µg of a mouse monoclonal antibody specific for human CD166; lyophilized.	
Anti-CD44 Purified Mouse Monoclonal IgG _{2a} Clone: 2C5	965614	25 µg of a mouse monoclonal antibody specific for human CD44; lyophilized.	
Anti-CD19 Purified Mouse Monoclonal IgG ₁ Clone: 4G7-2E3	965615	25 µg of a mouse monoclonal antibody specific for human CD19; lyophilized.	
Anti-CD45 Purified Mouse Monoclonal IgG ₁ Clone: 2D1	965616	25 µg of a mouse monoclonal antibody specific for human CD45; lyophilized.	
*Provided this is within 1 year from date of receipt.			

OTHER MATERIALS & 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

PRECAUTIONS

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

LIMITATIONS OF THE PROCEDURE

- 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.

REAGENT & MATERIAL 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.*

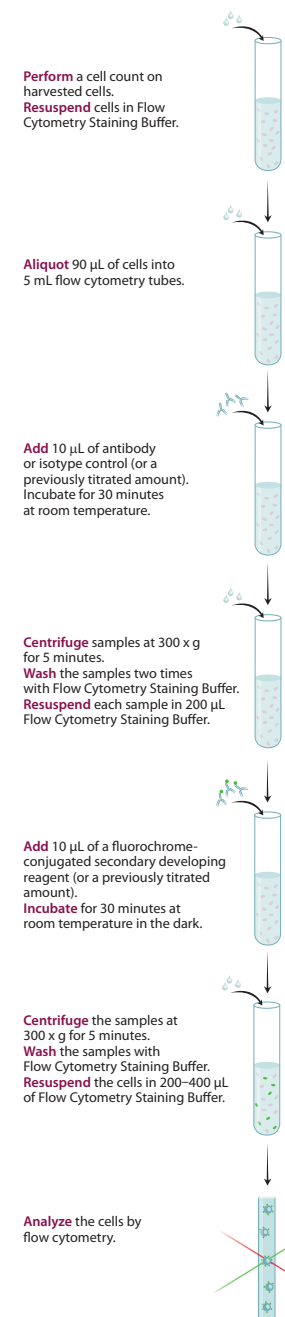
PROCEDURE

Note: *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.
Note: *As a control for analysis, cells in a separate tube should be treated with isotype control.*
3. Incubate for 30 minutes at room temperature.
4. Following incubation, pellet cells by centrifugation at 300 xg for 5 minutes. 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 room temperature **in the dark**.
7. Following incubation, pellet cells by centrifugation at 300 xg for 5 minutes. 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.

FLOW CYTOMETRY SURFACE STAINING OUTLINE



RELATED REAGENTS

Product Description	R&D Systems 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