MSC Characterization Antibody Panel

Multi-color flow cytometry kit for characterization of human mesenchymal stromal cells (MSCs)

Catalog #100-0354 1 Kit

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Product Description

The MSC Characterization Antibody Panel is designed for the phenotypic analysis of human mesenchymal stromal cells (MSCs). This kit contains three fluorochrome-conjugated antibodies for the identification of human MSCs, and a fluorochrome-conjugated antibody for the identification of non-MSCs. When measured by flow cytometry, ≥ 95% of the MSC population must express CD73, CD90, and CD105, and these cells must lack expression (≤ 2% positive) of CD45.[2, 3] These reagents provide single-step labeling for the verification of human MSCs. The MSC Characterization Antibody Panel can be used to identify human MSCs derived from various tissues such as bone marrow MSCs (BM-MSCs), pluripotent stem cell-derived mesenchymal progenitor cells (hPSC-MPCs), adipose-derived MSCs (ADSCs), dental pulp-derived MSCs (DPSCs), and umbilical cord-derived MSCs (UC-MSCs).

Product Information

The following components are sold as part of a complete kit (Catalog #100-0354) and are also available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE	FORMAT
Anti-Human CD73 Antibody, Clone AD2, PE	60044PE.1	125 µL	Store at 2 - 8°C. Do not freeze.	Protect product from prolonged exposure to light. For product expiry date, please contact techsupport@stemcell.com.	PBS, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA.
Anti-Human CD90 Antibody, Clone 5E10, FITC	60045FI.1	125 µL	Store at 2 - 8°C. Do not freeze.	Protect product from prolonged exposure to light. For product expiry date, please contact techsupport@stemcell.com.	PBS, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA.
Anti-Human CD105 (Endoglin) Antibody, Clone SN6h, APC	100-0243	125 µL	Store at 2 - 8°C. Do not freeze.	Stable until expiry date (EXP) on label.	PBS, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA.
Anti-Human CD45 Antibody, Clone HI30, PerCP-Cy5.5	60018PS.1	125 µL	Store at 2 - 8°C. Do not freeze.	Protect product from prolonged exposure to light. For product expiry date, please contact techsupport@stemcell.com.	PBS, pH 7.2, containing 0.09% sodium azide and 0.1% gelatin.

BSA - bovine serum albumin; PBS - phosphate-buffered saline

Materials Required But Not Included

PRODUCT NAME	CATALOG #
EasySep™ Buffer	20144
Anti-Human CD32 Antibody, Clone IV.3	60012
DAPI (Hydrochloride) OR DRAQ7™	75004 OR BioLegend 424001
OneComp eBeads™ Compensation Beads	Thermo Fisher 01-1111-42
Falcon® Round-Bottom Polystyrene Tubes, 5 mL OR Corning® 96-Well Round-Bottom Microplate	100-0088 OR 38018

Directions for Use

The MSC Characterization Antibody Panel is compatible with human MSCs cultured in MesenCult™-ACF Plus Medium (Catalog #05445/#05448), MesenCult™ Proliferation Kit (Human; Catalog #05411), or MesenCult™-hPL Medium Kit (Catalog #05439). Refer to the Product Information Sheet for MesenCult™-ACF Plus Medium (Catalog #05445/#05448) for a detailed protocol on culturing MSCs and detaching cells from cultureware for flow cytometry.
NOTE: Due to the low frequency of MSCs in human tissues, it is recommended to expand these cells in culture prior to flow cytometry analysis.

The following protocol is for preparing cells for flow cytometry analysis. Refer to Table 1 for a list of recommended conditions.
NOTE: This kit contains sufficient reagents to perform the following protocol approximately 83 times.

1. Add 10 mL of EasySep™ Buffer to detached MSCs and centrifuge at 300 x *g* for 7 minutes at room temperature (15 - 25°C). Discard supernatant and resuspend in a small amount of EasySep™ Buffer (e.g. 300 µL). Remove a small aliquot of cells and perform a cell count.
2. OPTIONAL: Add 1 µg of Anti-Human CD32 Antibody, Clone IV.3 (Fc blocker) per 1 x 10^6 cells to be labeled. Incubate at room temperature for 10 minutes. Proceed to next step.

   NOTE: An Fc blocker blocks non-specific binding of Fc receptor-expressing cells such as myeloid and B cells. An Fc blocker is not necessary with a highly pure MSC culture, as they do not possess Fc receptors. However, addition of Fc blocker is recommended when labeling MSC cultures that may contain contaminating hematopoietic cells (i.e. early passage MSCs).
3. To prepare heat-killed MSCs (Condition 6 in Table 1), transfer 0.5 - 3 x 10^5 cells in 90 µL of EasySep™ Buffer to a 5 mL round-bottom polystyrene tube labeled with Condition 6. Incubate at 65°C for 5 minutes in a water bath or incubator.
4. For other conditions requiring MSCs (as indicated in Table 1), transfer 0.5 - 3 x 10^5 cells in 90 µL of EasySep™ Buffer to 5 mL round-bottom polystyrene tubes labeled with Condition #.

   NOTE: If desired, a 96-well round-bottom plate (e.g. Catalog #38018) can be used instead of tubes. If using a plate, transfer the heat-killed MSCs (prepared in step 3) to the plate for Condition 6.
5. Prepare antibody master mixes corresponding to conditions 2 - 5 and 7 - 12 in Table 1, as follows:

   If labeling 90 µL of cells, dilute antibodies 1 in 40 in EasySep™ Buffer per condition.

   For example, dilute 2 µL of each antibody in a final volume of 80 µL of EasySep™ Buffer. When 10 µL of this antibody master mix is added to 90 µL of cells, the final antibody concentration will be 1 in 400.

   NOTE: Protect antibodies from prolonged exposure to light.

   NOTE: Using multiple fluorochromes requires proper flow cytometric compensation to remove the spillover fluorescence from a particular probe to a certain channel.[1] Conditions that use all reagents except for the one of interest (termed fluorescence minus one [FMO]) are necessary to accurately identify expressing cells in the fully labeled sample.[4]

   NOTE: A final antibody dilution of 1 in 400 is recommended when labeling 0.5 - 3 x 10^5 cells in 100 µL.
6. Add 10 µL of each Compensation Control antibody master mix (Conditions 2 - 6) to one of the following, as indicated by “Samples Labeled” in Table 1:
   - OneComp eBeads™ Compensation Beads (10 µL beads + 80 µL EasySep™ Buffer)

     OR
   - MSCs (prepared in steps 1 - 4)

   NOTE: Compensation beads are necessary for compensating CD45-PerCP-Cy5.5 (Condition 2) since MSCs should not express this marker. For compensation of CD73, CD90, and CD105, either compensation beads or MSCs can be used (Conditions 3 - 5).
7. Add 10 µL of each FMO antibody master mix (Conditions 7 - 11) to cells prepared in step 4.
8. Add 10 µL of MSC Characterization Antibody Panel master mix (Condition 12) to cells prepared in step 4.
9. Incubate the tubes or plate at 2 - 8°C for 30 minutes. Protect from light.
10. If using 5 mL tubes, add 3 mL of EasySep™ Buffer per tube. Centrifuge at 300 x *g* for 7 minutes at room temperature. Remove and discard supernatant.

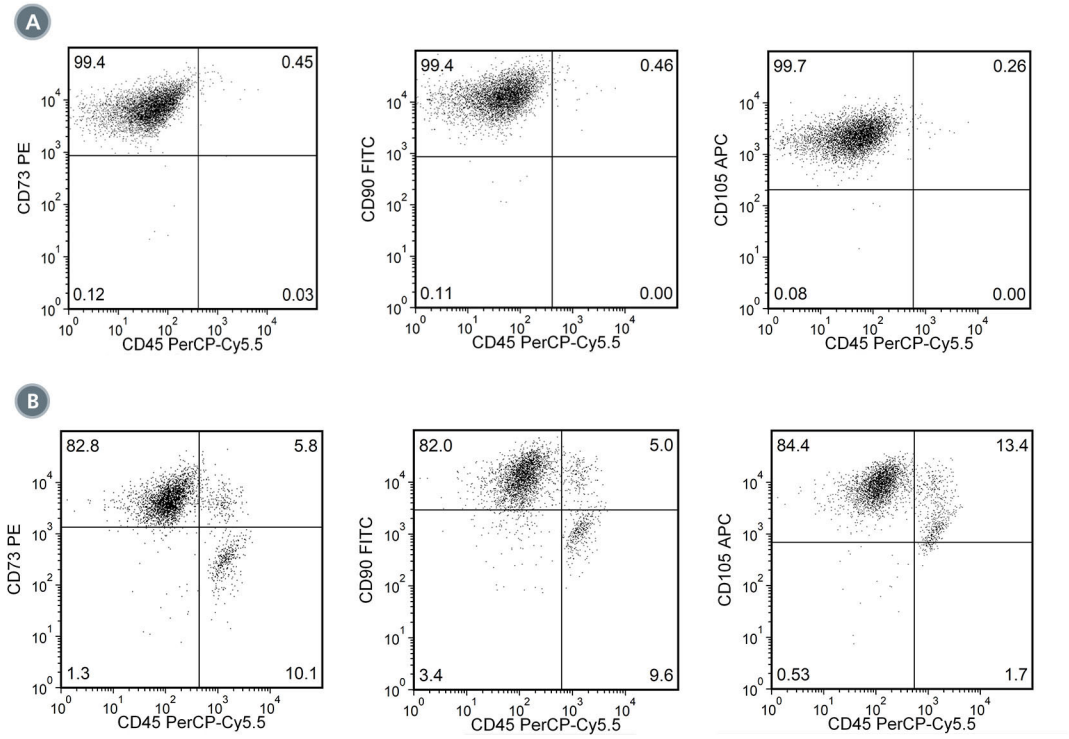
    OR

    If using a 96-well plate, add 100 µL of EasySep™ Buffer per well. Centrifuge at 700 x *g* for 3 minutes at room temperature. Remove and discard supernatant.
11. Repeat step 10.
12. Resuspend cells in EasySep™ Buffer as indicated below. If viability stain is indicated in Table 1, add DAPI or DRAQ7™ to the EasySep™ Buffer at a 1 in 5000 dilution. Mix thoroughly.
    - For 5 mL tubes: 200 - 400 µL of EasySep™ Buffer
    - For 96-well plate: 100 - 200 µL of EasySep™ Buffer
13. Cells are ready for analysis by flow cytometry.

Table 1: Conditions for Flow Cytometry Analysis

CONDITION #	TYPE OF TEST	ANTIBODIES	SAMPLES LABELED	VIABILITY STAIN ADDED
1	Unstained Control	None	MSCs	No
2	Compensation Control	CD45-PerCP-Cy5.5	Compensation Beads	No
3	Compensation Control	CD73-PE	Compensation Beads or MSCs	No
4	Compensation Control	CD90-FITC	Compensation Beads or MSCs	No
5	Compensation Control	CD105-APC	Compensation Beads or MSCs	No
6	Compensation Control	Viability stain: DAPI or DRAQ7™	Heat-killed MSCs	Yes
7	FMO: Minus CD45	CD73-PE + CD90-FITC + CD105-APC	MSCs	Yes
8	FMO: Minus CD73	CD45-PerCP-Cy5.5 + CD90-FITC + CD105-APC	MSCs	Yes
9	FMO: Minus CD90	CD45-PerCP-Cy5.5 + CD73-PE + CD105-APC	MSCs	Yes
10	FMO: Minus CD105	CD45-PerCP-Cy5.5 + CD73-PE + CD90-FITC	MSCs	Yes
11	FMO: Minus Viability Stain	CD45-PerCP-Cy5.5 + CD73-PE + CD90-FITC + CD105-APC	MSCs	No
12	MSC Characterization Antibody Panel	CD45-PerCP-Cy5.5 + CD73-PE + CD90-FITC + CD105-APC	MSCs	Yes

Data



(A) When cultured in MesenCult™-ACF Plus Medium, cells demonstrate expression of CD73, CD105, and CD90 and do not express CD45 at P0.

(B) When cultured in serum-containing medium, cells exhibit reduced CD73, CD105, and CD90 as well as increased CD45 expression at P0 due to contaminating CD45+ cells.

References

1. Bagwell CB & Adams EG. (1993) Fluorescence spectral overlap compensation for any number of flow cytometry parameters. Ann N Y Acad Sci 677(1): 167–184.

2. Bourin P et al. (2013) Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: A joint statement of the International Federation for Adipose Therapeutics and Science (IFATS) and the International Society for Cellular Therapy (ISCT). Cytotherapy 15(6): 641–8.

3. Dominici M et al. (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8(4): 315–7.

4. Roederer M. (2001) Spectral compensation for flow cytometry: visualization artifacts, limitations, and caveats. Cytometry 45(3): 194–205.