Human Mesenchymal Stromal Cell kit

Catalog Number：ICA-A006

Size: 50 tests

IMPORTANT: Please carefully read this user guide before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

Product information

Human multipotent mesenchymal stromal cells (MSCs), often referred to as mesenchymal stem cells, represent a rare subset of adult stem cells that can be isolated from multiple tissue sources. When derived from bone marrow and expanded in culture, MSCs are capable of differentiating into a range of cell types, most prominently adipocytes, osteocytes, and chondrocytes. Beyond their differentiation potential, MSCs exhibit immunomodulatory properties both in vitro and in vivo.

In 2006, the International Society for Cellular Therapy (ISCT) established a minimal set of criteria for the identification of human bone marrow–derived MSCs. According to these guidelines, MSCs should express CD73, CD90, and CD105, while lacking expression of CD34, CD45, CD11b or CD14, CD19 or CD79α, and HLA-DR. MSCs are also known to express additional markers, including CD44, CD29, CD200, CD166, CD146, and CD271.

This kit incorporates the ISCT-recommended panel and is designed with a modular layout that allows users to add extra markers as needed. The MSC positive cocktail—comprising PerCP-Cy5.5-labeled CD90, PE-CY7-labeled CD105, and APC-labeled CD73—keeps the PE channel available, allowing it to be used alongside the negative MSC cocktail (PE-labeled CD45, CD34, CD11b, CD19, and HLA-DR), the included PE CD44 antibody, or other PE-conjugated antibodies from commercial sources. This multicolor approach reduces the number of cells required per assay, while the antibody cocktails simplify the staining workflow for simultaneous analysis of multiple samples. Single-stain positive controls are provided to facilitate compensation setup. Greater than or equal to 95% of the MSC population must express CD105, CD73 and CD90 as measured by flow cytometry. Additionally, these cells must lack expression (≤2% positive) of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DR.

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Components

Components	Size	Format
APC-Labeled Monoclonal Anti-Human CD73 Antibody, Mouse IgG1 (5A10)	50tests	Liquid
PerCP-Cy5.5 Labeled Monoclonal Anti-Human CD90 Antibody, Mouse IgG1 (AS677)	50tests	Liquid
PE-CY7-Labeled Monoclonal Anti-Human CD105 Antibody, Mouse IgG1 (AS682)	50tests	Liquid
Positive Cocktail	50tests	Liquid
PE-Labeled Monoclonal Anti-Human CD44 Antibody, Mouse IgG1 (AS625)	50tests	Liquid
PE-Labeled Monoclonal Anti-Human CD11b Antibody, Mouse IgG1 (AS673)	50tests	Liquid
PE-Labeled Monoclonal Anti-Human CD19 Antibody, Mouse IgG2a (FMC63)	50tests	Liquid
PE-Labeled Monoclonal Anti-Human CD34 Antibody, Mouse IgG1 (AS579)	50tests	Liquid
PE-Labeled Monoclonal Anti-Human CD45 Antibody, Mouse IgG2a (2E9)	50tests	Liquid
PE-Labeled Monoclonal Anti-Human HLA-DR Antibody, Mouse IgG2a (L243)	50tests	Liquid
Positive Isotype Control Cocktail	50tests	Liquid
Negative Isotype Control Cocktail	50tests	Liquid
PE-Labeled Mouse IgG1 Antibody Isotype Control	50tests	Liquid

Storage

Keep the unopened kit at 2-8 °C. Avoid using the kit beyond its expiration date.

The opened kit and the reagents should be stored at 2-8 °C protected from light and freeze-thaw cycles.

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Required Materials not Supplied

Instrument	Multi-channel flow cytometer: the equipment configuration requirements are detailed in the following table *"List of Compatible Flow Cytometers"* in page 6
	Vortex oscillator
	Centrifuge with a 96-well plate rotator (for a 96-well plate samples preparation)
	Cell counter
Reagents	Deionized, ultrapure or distilled water
	1×PBS
	BSA
Consumables	12×75 mm Flow tubes
	96-well Plate (flow instrument support 96-well plate testing and samples preparation for an auto-samples running)
	Pipette suitable for 2 µL, 10 µL, 100 µL, 1 mL and disposable pipette tip.

List of Compatible Flow Cytometers

Manufacturer	Instrument model	Laser filter (nm)	Em Filter and Fluorochrome
Miltenyibiotec	MACSQuant Analyzer 10	405, 488, 561, 633	B4-PE-CY7, B3-PerCP-CY5.5, B2-PE, R1-APC
Beckman Coulter	Cytoflex S	405, 488, 561, 638	Y780-PE-CY7, B690-PerCP-CY5.5, Y585-PE, R660-APC
BD	FACS Lyric	405, 488, 640	PE-CY7, PerCP-CY5.5, PE, APC
	FACSymphony A1	405, 488, 561, 637	PE-CY7, BB515-PerCP-CY5.5, APC, PE
Thermo	Attune NxT	405, 488, 561, 637	YL3-PE-CY7, BL3-PerCP-CY5.5, RL1-APC, YL1-PE

Precaution

1. Use before the expiration date on the kit label.
2. Keep fluorochrome-conjugated antibodies protected from light.
3. Do not mix or substitute reagents from different lots or sources.
4. All chemicals should be considered as potentially hazardous. It is recommended that this kit is handled only by those people who have been trained in laboratory techniques and it is used in accordance with the principles of good laboratory practice. Suitable protective clothing such as laboratory overalls, safety glasses and gloves is needed. Attention should be paid to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with plenty of water. All blood components and biological materials should be handled and disposed properly, in accordance with local and national guidelines.

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Working Buffers Preparation

Staining buffer (1% BSA, 1×PBS): 0.5 g of BSA was dissolved in 50 mL of 1×PBS, which can be regulated according to the samples used in your experiment.

Procedure

1. Harvest MSCs and resuspend in an appropriate volume of Staining buffer (for example, 6–10 mL buffer for 1×10^7 cells), centrifuge at 300 g for 5 minutes and discard the supernatant.

   Note:

   This kit is applicable to both fresh and cryopreserved cell samples.

2. Resuspend the cell pellet in an appropriate volume of Staining buffer (for example, 3–5 mL buffer for 1×10^7 cells) and count the cells with a cell counter.

   Pipette 5×10^5 cells for each sample, centrifuge at 300 g for 5 minutes and remove the supernatant. Resuspend the cells with a mixture of 95 μL staining buffer and 5 μL fluorescent antibody, then mix gently. **Label tubes and add antibodies as shown below:**

   Note:

   1. *The recommended detection range of cells for this kit is from 2×10^5 to 1×10^6.*
   2. *Tube 1-6 only need to be processed once for compensation adjustment. Additional cell samples shall be tested in Tube 7-10.*

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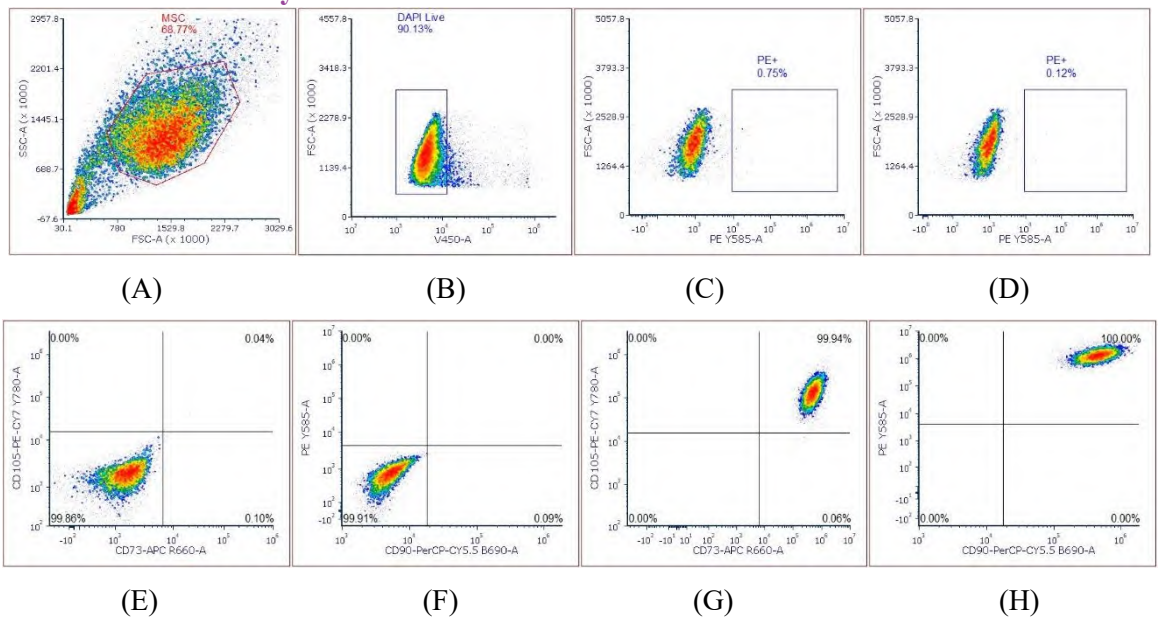
Tube Number	Experimental Group	Sample Name	Buffer Volume (μL)	Sample Volume (μL)
1	Blank Control Group	Blank	100 μL	NA
2	Viability & Dead Cell Group	DAPI	100 μL	NA
3	Single Positive Tube (for compensation adjustment)	CD73-APC	95 μL	5 μL
4		CD90-PerCP-CY5.5	95 μL	5 μL
5		CD105-PE-CY7	95 μL	5 μL
6		CD44-PE	95 μL	5 μL
7	Positive Marker Isotype Control Group	Positive Isotype Control Cocktail (Optional: + Mouse IgG1-PE for CD44-PE)	90 μL	5 μL (Optional: 5 μL)
8	Negative Marker Isotype Control Group	Negative Isotype Control Cocktail	95 μL	5 μL
9	Positive Experimental Group	Positive Cocktail (Optional: + CD44-PE)	90 μL	5 μL (Optional: 5 μL)
10	Negative Experimental Group	CD11b-PE+CD19-PE+CD34-PE+CD45-PE+HLA-DR-PE	75 μL	5 μL+5 μL+5 μL+5 μL+5 μL

3. Incubate for 15 min in the dark on ice, centrifuge for 5 min at 300 g. Discard the supernatant.
4. Resuspend cells with 250 μL of Staining buffer and centrifuge for 5 min at 300 g. Discard the supernatant.
5. Repeat the washing step 4 once.
6. Except blank control, resuspend cells in DAPI live/dead staining solution preformulated with staining buffer at a volume of 250 μL prior to detection and collect within 10 min. If samples are not to be analyzed immediately, store them with staining buffer only in the dark on ice.

   ***Note**:*

   1. *For the overlap interruption of the spectrum Propidium Iodide (PI) and 7-AAD are not suggested cell staining for the usage of this kit.*
   2. *Add DAPI to stained cell samples prior to instrument analysis, test promptly to prevent DAPI-induced cellular damage.*
7. *Analyze the cells on a flow cytometer, using Tube 1 for baseline setting, Tube 2 for DAPI single staining and Tubes 3-6 for antibody single staining to adjust compensation.*

Representative Data Analysis



(A) (B) (C) (D)

(E) (F) (G) (H)

MSCs analysis

An FSC/SSC scatter plot was established for the MSC and gate as P1 (A). As MSCs with the P1 gate, set the P2 gates with DAPI /SSC scatter plot that assessed the viable cell population (B). As living cell population with DAPI gate, set the Y585-A/SSC scatter plot to assess the negative isotype control (C) and set the Y585-A/SSC scatter plot to assess the negative cocktail (CD11b, CD19, CD34, CD45, HLA-DR) (D). Similarly, select the DAPI gate, set the CD105-PE-CY7/CD73-APC positive isotype control scatter plot (E) and set the CD90-PerCP-CY5.5/CD44-PE positive isotype control scatter plot (F). Correspondingly, set the CD105-PE-CY7/CD73-APC scatter plot as positive cocktail (G) and set the CD90-PerCP-CY5.5/CD44-PE scatter plot as positive cocktail(H).

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