Human MSC Analysis Kit

Cat. No: XJH003 **Size: 10 Assays/50 Assays/200 Assays**

Kit Components

Cat	Products	10 Assays	50 Assays	200 Assays	Storage
XJH003A	Human MSC Positive and Negative Marker Cocktail	100 μL	500 μL	1 mL×2	2-8°C, shading light
XJH003B	Human MSC Isotype Controls Cocktail	100 μL	500 μL	1 mL×2	
E-AB-F1167C	FITC Anti-Human CD90 Antibody[5E10]	50 μL	250 μL	500 μL×2	
E-AB-F1242D	PE Anti-Human CD73 Antibody[AD2]	50 μL	250 μL	500 μL×2	
E-AB-F1310E	APC Anti-Human CD105 Antibody[SN6]	50 μL	250 μL	500 μL×2	
E-AB-F1215Q	Elab Fluor®Violet 450 Anti-Human CD44 Antibody[Hermes-1]	50 μL	250 μL	500 μL×2	
	Manual	1 copy			

Composition of Components

Products	Component
Human MSC Positive and Negative Marker Cocktail	Elab Fluor®Violet 450 Anti-Human CD45 Antibody[HI30]
	Elab Fluor®Violet 450 Anti-Human CD34 Antibody[581]
	Elab Fluor®Violet 450 Anti-Human CD14 Antibody[H332-1B10]
	Elab Fluor®Violet 450 Anti-Human CD19 Antibody[SJ25C1]
	Elab Fluor®Violet 450 Anti-Human HLA-DR Antibody[L243]
	FITC Anti-Human CD90 Antibody[5E10]
	PE Anti-Human CD73 Antibody[AD2]
	APC Anti-Human CD105 Antibody[SN6]
Human MSC Isotype Controls Cocktail	Elab Fluor®Violet 450 Mouse IgG1, κ Isotype Control[MOPC-21]
	Elab Fluor®Violet 450 Rat IgG1, κ Isotype Control[HRPN]
	Elab Fluor®Violet 450 Mouse IgG2a, κ Isotype Control[C1.18.4]
	FITC Mouse IgG1, κ Isotype Control[MOPC-21]
	PE Mouse IgG1, κ Isotype Control[MOPC-21]
	APC Mouse IgG1, κ Isotype Control[MOPC-21]

Note: It is not recommended to mix Cocktail from different batches of kits.

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Storage

Store at 2-8°C with shading light for 2 years. Avoid freezing and thawing.

Description

This kit is used for the identification of human mesenchymal stem cells (hMSCs). The International Society for Cellular Therapy (ISCT) defines mesenchymal stem cells (MSCs) as adult stem cells with self-renewal and multi-lineage differentiation potential (such as osteogenic, chondrogenic, and adipogenic differentiation), characterized by plastic-adherent growth. They hold great application prospects in regenerative medicine, tissue engineering, and immunotherapy. According to flow cytometry detection, MSCs must co-express CD90, CD73, and CD105 at high levels (≥95%) and must lack expression (≤2% positive) of CD45, CD34, CD14 or CD11b, CD19 or CD79α, HLA-DR .

MSCs are also known to express numerous cell surface markers, such as CD44, CD29, CD200, CD166, CD146 and CD271. In this kit, we include the combination proposed by ISCT. Specifically, MSC positive and negative indicators cocktail(FITC CD90, PE CD73, APC CD105, Elab Fluor®Violet 450 CD45, CD34, CD14, CD19, HLA-DR), Isotype control corresponding to MSC positive and negative indicators cocktail(FITC Mouse IgG1, κ; PE Mouse IgG1, κ; APC Mouse IgG1, κ; Elab Fluor®Violet 450 Mouse IgG1, κ, Rat IgG1, κ, Mouse IgG2a, κ), and also contains separate positive markers for compensation Settings.

The Human MSC Analysis Kit enables rapid verification of hMSC purity, screen cell lines that meet experimental requirements, and significantly shorten the detection cycle. This kit has been validated for the evaluation of umbilical cord-derived hMSCs expanded under common MSC culture conditions.

Reagents and Materials Not Supplied

1. **Reagents:**

   PBS, human mesenchymal stem cell serum-free culture medium, 0.25% trypsin solution (containing EDTA, dissolved in PBS)

2. **Materials:**

   70 μm mesh nylon strainer, 1.5 mL/2 mL EP tube, 15 mL/50 mL centrifuge tube, flow tube

3. **Instrument:**

   Optical microscope, CO_2 incubator, horizontal centrifuge, flow cytometer

Experimental Operation

a) Collect the cell suspension, centrifuge at 300×g for 5 min, completely discard the supernatant, and resuspend the cells in an appropriate amount of PBS (1×).

b) Count cells and adjust the cell suspension concentration ($1×10^7$ cells/mL)

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c) According to the experimental design, group the samples as specified in the table below, with 100 µL of cell suspension per tube. (If the cell quantity is insufficient, the cell count in the single-staining tube used for adjustment and compensation can reduced to 5×10^5 cells per tube.)

Tube	Sample tube setup	Purpose	E-AB-F1167C	E-AB-F1242D	E-AB-F1310E	E-AB-F1215Q	XJH003A	XJH003B
1	Blank	Adjust voltage						
2	CD90	Adjust compensation	✔					
3	CD73	Adjust compensation		✔				
4	CD105	Adjust compensation			✔			
5	CD44	Adjust compensation				✔		
6	MSC whole	MSC identify					✔	
7	MSC Isotype control	Remove background staining						✔

d) Except for Blank tube, 1 Assay (5 µL/Assay) of antibody was added to Tubes 2 to 5. 1 Assay (10 µL/Assay) of antibody Cocktail was added to Tubes 6 and 7. Mix thoroughly and incubate in the dark at 4 °C for 25-30 min.

e) After incubation, add 1 mL of PBS (1×), mix well, and centrifuge at 300×g for 5 min, then discard the supernatant.

f) Resuspend the cells in 200 µL PBS (1×), then filter the cell suspension through a 70 µm cell sieve, and perform detection and analysis using a flow cytometer.

Note:

✧ The voltage and threshold settings of the flow cytometer must be calibrated prior to formal data acquisition. Voltage and threshold adjustments are prohibited after initiating signal collection.

✧ Criteria for identifying human umbilical cord mesenchymal stem cells: The final determination must meet both core indicators simultaneously: ① The proportion of $CD90^+$ $CD73^+$ $CD105^+$ cells ≥95%; ② The proportion of $CD14^+$ $CD19^+$ $CD34^+$ $CD45^+$ HLA-DR^+ cells ≤2%.

Typical data

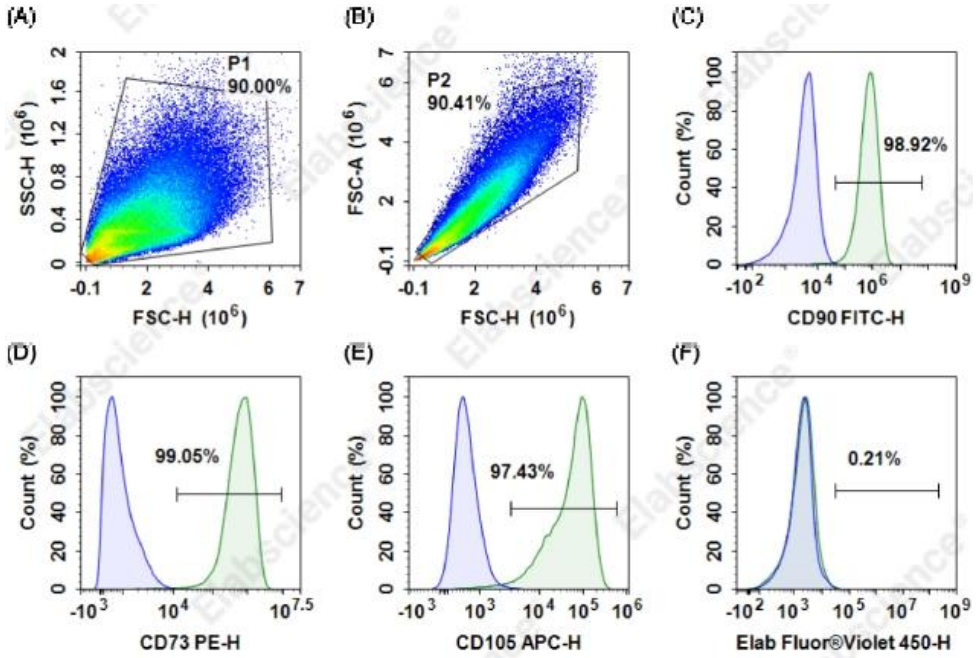

Figure 1. (A) From FSC-H/SSC-H, a threshold (P1) was set to exclude debris.(B) Within the P1 threshold, FSC-H/FSC-A was used to set another threshold (P2) to remove adherent cells. Within the P2 threshold, the expression levels of three positive markers and negative markers were analyzed. As shown in the figure, (C) the proportion of $CD90^+$ cell population was 98.92%, (D) the proportion of $CD73^+$ cells was 99.05%, (E) the proportion of $CD105^+$ cell population was 97.43%, and (F) the proportion of $CD14^+$ $CD19^+$ $CD34^+$ $CD45^+$ $HLA\text{-}DR^+$ cells was 0.21%, indicating that these cells were identified as human mesenchymal stem cells.

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. All components of the kit should be stored at 2-8°C, protected from light, and avoid freezing and thawing.
4. The sample used for product demonstration data is human umbilical cord mesenchymal stem cells, with a concentration of 1×10^6 cells/Assay.
5. The cell suspension must be filtered through a 70 μm cell sieve to remove cell clumps, preventing aggregation that could compromise identification purity.
6. In order to ensure the activity of the cells, the whole process of the experiment should be completed on ice as much as possible.

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