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50 µL CD90-FITC, human (clone: DG3, isotype: mouse IgG1)

50 µL CD105-PE, human (clone: 43A4E1, isotype: mouse IgG1)

50 µL CD73-APC, human (clone: AD2, isotype: mouse IgG1)

50 µL CD73-Biotin, human (clone: AD2, isotype: mouse IgG1)

50 µL Anti-Biotin-PerCP, (clone: Bio3-18E7, isotype: mouse IgG1)

Capacity

50 tests or up to 2×10⁸ total cells.

Product format

Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage

Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components 500 µL MSC Phenotyping Cocktail, human:
Cocktail of fluorochrome-conjugated monoclonal antibodies:
CD14-PerCP (clone: TÜK4, isotype: mouse IgG2a),
CD20-PerCP (clone: LT20.B4, isotype: mouse IgG1),
CD34-PerCP (clone: AC136, isotype: mouse IgG2a),
CD45-PerCP (clone: 5B1, isotype: mouse IgG2a),
CD73-APC (clone: AD2, isotype: mouse IgG1),
CD90-FITC (clone: DG3, isotype: mouse IgG1),
CD105-PE (clone: 43A4E1, isotype: mouse IgG1).

500 µL Isotype Control Cocktail, human:
Cocktail of fluorochrome-conjugated monoclonal antibodies:
Mouse IgG1-FITC (clone: IS5-21F5),
Mouse IgG1-PE (clone: IS5-21F5),
Mouse IgG1-APC (clone: IS5-21F5),
Mouse IgG1-PerCP (clone: IS5-21F5),
Mouse IgG2a-PerCP (clone S43.10).

1.1 Background information

Mesenchymal stem cells or stromal cells (MSCs) are of great interest for therapeutic use in clinical applications. Also, MSCs are isolated from a variety of tissues, expanded under different conditions, and characterized with broad variety. Minimal criteria for a common definition of human MSCs were defined by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) to facilitate a better comparability of data amongst investigators, accelerating new scientific discoveries, and facilitating the development of novel cellular therapies.¹ They proposed that MSCs should be plastic-adherent when maintained under standard culture conditions. Also MSCs should differentiate to osteoblasts, adipocytes, and chondrocytes under standard *in vitro* differentiating conditions. 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 CD34, CD45, CD11b or CD14, CD19 or CD79a, and HLA class II.

Marker	Expressed by
MSC-positive marker	
CD73, CD90, CD105	MSCs
MSC-negative marker	
CD14	Monocytes and macrophages
CD20	Pan B cells (comparable to CD19 and CD79a)
CD34	Primitive hematopoietic progenitors and endothelial cells
CD45	Pan-leukocyte cells

The MSC Phenotyping Kit contains ready-to-use antibody cocktails that reliably identify human MSCs, as well as single antibodies for the compensation of the flow cytometer, e.g., the MACSQuant® Analyzer. The MSC Phenotyping Kit enables the fast, easy, and reliable phenotyping of cultured MSCs based on the defined ISCT standards.¹

1.2 Applications

- Identification and phenotyping of cultured MSCs based on the defined ISCT standards¹ by flow cytometry.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

MSC Phenotyping Cocktail and Isotype Control Cocktail	
Flow cytometry ^a	1:11
- In general	

a) The indicated antibody dilutions are for up to 10⁶ cells/100 µL of buffer.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
 - ▲ Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- Flow cytometer, e.g., MACSQuant[®] Analyzer 10 (# 130-096-343)
- (Optional) StemMACS[™] MSC Expansion Media Kit XF, human (# 130-104-182) for expansion of MSCs.

2. Protocols

2.1 Immunofluorescent staining

▲ The following protocol is for working with cultured human MSCs. A special protocol for isolation, expansion, and phenotyping of MSCs is available at www.miltenyibiotec.com/applications.

▲ The flow cytometer should be compensated for MSCs. For a compensation protocol refer to 5. Appendix.

▲ Cultured MSCs have to be dissociated with a trypsin/EDTA solution and used immediately.

▲ Volumes given below are for up to 10⁶ nucleated cells. When working with fewer than 10⁶ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10⁶ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Take two aliquots (1 and 2), each with up to 1×10⁶ cells.
3. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
4. Resuspend each aliquot in 100 µL of buffer.
5. Add 10 µL of the MSC Phenotyping Cocktail into aliquot 1 and 10 µL of the Isotype Control Cocktail into aliquot 2.

6. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

7. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

▲ Note: Make sure that the flow cytometer is optimally compensated for the analysis of MSCs.

2.2 Fully automated flow analysis with the MACSQuant[®] Analyzer 10 using the Express Mode

▲ For fully automated flow cytometric analysis of MSCs with the MACSQuant[®] Analyzer 10, use the MSC_Phenotyping_Kit_h Express Mode function. For details of how to use the Express Modes, please refer to the MACSQuant Instrument user manual, the MACSQuantify[™] Software guide, or visit www.miltenyibiotec.com/macsqantify.

The Express Modes have to be performed as **grouped acquisition and analysis**. By selecting the Express Mode all experiment settings are automatically loaded. The loaded values are shown in the respective fields in the **Experiment** tab. These can be adapted manually if needed, for example, uptake volume.

Grouping of samples required?	Yes
2 sample IDs pre-defined by the Express Mode	Isotype Control MSC Phenotyping

1. Prepare and prime the MACSQuant Analyzer 10. Make sure the calibration and compensation settings have been optimized for acquisition of the MSC Phenotyping Cocktail and are loaded by selecting **File – Open**. For PerCP, PE, APC, and FITC compensation use the single antibodies provided within the kit.
2. Select the **Chill 5 Rack** of the rack drop-down list.
3. Define sample positions. Therefore mark the rack positions that containing samples by clicking on A1 (Isotype Control) and B1 (MSC Phenotyping). Group the desired sample positions by using the **Group** button. Grouped samples will be assigned by the same group number.
 - ▲ Note: Do not group more positions than sample IDs available. Only rack positions that are adjacent and in columns can be grouped.
4. Load the Express Mode by selecting the **Settings** tab.
5. Check the **Express** button.
6. Select **Analysis** from the **Type** drop-down list.
7. Choose the Express Mode **MSC_Phenotyping_Kit_h** from the **Mode** drop-down list.
 - ▲ Note: Optimal Express Mode analysis requires hlog setting for the fluorochrome channels. For details refer to the the MACSQuantify[™] Software guide.
8. Enter a description for each grouped sample set for better assignment of different experiments.
9. Start the measurement. Express Mode analysis is automatically performed, after all sample positions of a group has been acquired.
10. For the analysis of data files on a personal computer right-click within the **Samples** tab and select **Open...** or **Add...** from the context menu to add data files to the MACSQuantify[™] Software.

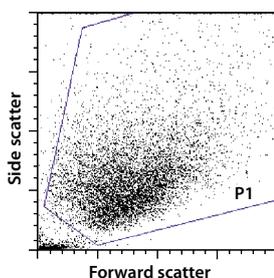
11. Navigate to desired data files, select them, and open them into the software.
12. Right-click on the file name and select **View with express Analysis. MSC_Phenotyping_Kit_h** for accessing the Express Mode analysis template. The data will be displayed in an analysis window.
13. The selection has to be repeated for each analyzed file.

3. Examples of immunofluorescent staining and gating strategy with the MSC Phenotyping Kit

Aliquot 1 of cultured MSCs was stained with the MSC Phenotyping Cocktail and a aliquot 2 with the Isotype Control Cocktail. The fractions were analyzed by flow cytometry using the MACSQuant® Analyzer.

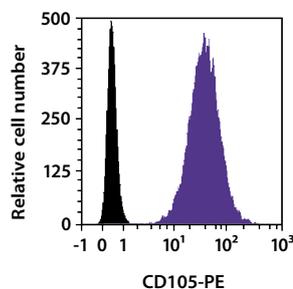
1. Take aliquot 2 (Isotype Control Cocktail).
2. Create a dot plot with forward scatter (FSC) versus side scatter (SSC).
3. Draw population 1 (P1) to exclude debris and platelets.

A)

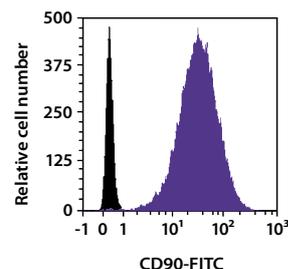


4. Using the P1-gated population create a histogram for each fluorescence channel: FL-2, FL-3, FL-4, and FL-6.
5. Apply these gate to aliquot 1 and 2 and create an overlay for each of the following fluorescence channels:
 - B) FL-3 – analysis of the CD105-PE⁺ cells
 - C) FL-2 – analysis of the CD90-FITC⁺ cells
 - D) FL-6 – analysis of the CD73-APC⁺ cells
 - E) FL-4 – analysis of the PerCP⁺ cells

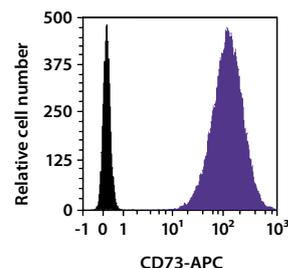
B)



C)

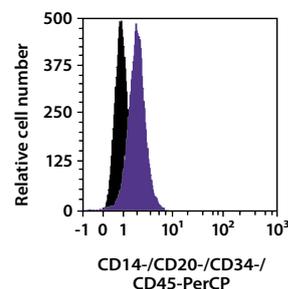


D)



6. As a control step, analyze the overlay histogram of FL-4. The MSCs should be negative for the hematopoietic markers.

E)



4. Reference

1. Dominici, M. *et al.* (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 8: 315–317.

5. Appendix

Compensation of flow cytometer

1. Determine cell number.
2. Take five aliquots (PerCP, PE, APC, FITC, blank), each with up to 5×10^5 MSCs.
3. Centrifuge cell suspension at $300 \times g$ for 10 minutes. Aspirate supernatant completely
4. Resuspend aliquots PerCP, PE, APC, and FITC in 100 μ L of buffer, respectively.
5. Resuspend aliquot blank in 500 μ L of buffer. This aliquot is ready-to-use. Proceed directly with compensation.
6. Add 10 μ L of CD73-Biotin in aliquot PerCP, CD105-PE in aliquot PE, 10 μ L of CD73-APC in aliquot APC, and 10 μ L of CD90-FITC in aliquot FITC.
7. Mix each aliquot well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
8. Wash each aliquot by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
9. Add 10 μ L of Anti-Biotin-PerCP to aliquot PerCP. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
10. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
11. Resuspend each cell pellet separately in 500 μ L buffer.
12. Compensate instrument by following the instructions in the instrument user manual.

All protocols and data sheets are available at www.miltenyibiotec.com.

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