MSC Phenotyping Cocktail Kit, anti-human, REAfinity™

Order no. 130-125-285

Contents

1. Description

This product is for research use only.

Components

0.5 mL MSC Phenotyping Cocktail, anti-human, REAfinity containing:

CD14 Antibody, anti-human, PE, REAfinity (clone: REA599, isotype: recombinant human IgG1)

CD19 Antibody, anti-human, PE, REAfinity (clone: REA675, isotype: recombinant human IgG1)

CD34 Antibody, anti-human, PE, REAfinity (clone: REA1164, isotype: recombinant human IgG1)

CD45 Antibody, anti-human, PE, REAfinity (clone: REA747, isotype: recombinant human IgG1)

CD73 Antibody, anti-human, APC, REAfinity (clone: REA804, isotype: recombinant human IgG1)

CD90 Antibody, anti-human, FITC, REAfinity (clone: REA897, isotype: recombinant human IgG1)

CD105 Antibody, anti-human, VioBlue®, REAfinity (clone: REA794, isotype: recombinant human IgG1)

HLA-DR Antibody, anti-human, VioGreen™, REAfinity (clone: REA805, isotype: recombinant human IgG1)

0.5 mL MSC Control Cocktail, anti-human, REAfinity containing:

REA Control Antibody (S), human IgG1, FITC, REAfinity (clone: REA293)

REA Control Antibody (S), human IgG1, PE, REAfinity (clone: REA293)

REA Control Antibody (S), human IgG1, APC, REAfinity (clone: REA293)

REA Control Antibody (S), human IgG1, VioBlue, REAfinity (clone: REA293)

REA Control Antibody (S), human IgG1, VioGreen, REAfinity (clone: REA293)

0.1 mL CD73 Antibody, anti-human, APC, REAfinity (clone: REA804, isotype: recombinant human IgG1)

0.1 mL CD73 Antibody, anti-human, PE, REAfinity (clone: REA804, isotype: recombinant human IgG1)

0.1 mL CD90 Antibody, anti-human, FITC, REAfinity (clone: REA897, isotype: recombinant human IgG1)

0.1 mL CD105 Antibody, anti-human, VioBlue, REAfinity (clone: REA794, isotype: recombinant human IgG1)

0.1 mL CD73 Antibody, anti-human, Biotin, REAfinity (clone: REA804, isotype: recombinant human IgG1)

0.1 mL Biotin Antibody, anti-human, VioGreen, REAfinity (clone: REA746, isotype: recombinant human IgG1)

Capacity

50 tests or up to 5×10^8 total cells.

Product format

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

Storage

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

1.1 Background information

Human mesenchymal stem cells or stromal cells (MSCs) hold great promise for regenerative applications like bone and cartilage repair, as well as for immunomodulatory applications. However, MSCs can be isolated from a variety of tissues, expanded under different conditions, and characterized with regard to differentiation potential as well as cell surface marker expression. Minimal criteria for a common definition of human MSCs were defined in 2006 by 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. The committee proposed that MSCs should be plastic-adherent when maintained under standard culture conditions. Also, MSCs should differentiate into osteoblasts, adipocytes, and chondrocytes under standard *in vitro* differentiation 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 CD79α, and HLA-DR.

The MSC Phenotyping Cocktail Kit, anti-human, REAfinity

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enables the fast, easy, and standardized phenotyping of cultured MSCs based on the following markers.

Marker	Expressed by
MSC-positive marker	
CD73, CD90, CD105	MSCs
MSC-negative marker	
CD14	Monocytes and macrophages
CD19	Pan B cells
CD34	Primitive hematopoietic progenitors and endothelial cells
CD45	Pan-leukocyte cells
HLA-DR	Dendritic cells, B cells, monocytes, macrophages

HLA-DR is constitutively expressed on professional antigen-presenting cells like dendritic cells, B cells, and monocytes/macrophages. On T cells, natural killer (NK) cells, hematopoietic precursor cells, and some epithelial cells, the expression of HLA-DR is induced by cell activation. On MSCs, HLA-DR is induced after stimulation.

The kit applies recombinantly engineered REAfinity Antibodies. REAfinity Antibodies are recombinant antibodies that provide superior lot-to-lot consistency and purity compared to mouse or rat hybridoma-derived, monoclonal antibodies. They have been recombinantly engineered to produce highly specific antibodies that require no FcR blocking step. Additionally, they all have the same IgG1 isotype, requiring less isotype controls.

1.2 Applications

- Identification and phenotyping of cultured human MSCs based on the defined ISCT standards regarding marker expression analyzed by flow cytometry.

1.3 Reagent and instrument 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 to +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 Ca^{2+} or Mg^{2+} are not recommended for use.

- Flow cytometer equipped with a red (640 nm), a blue (488 nm), and a violet (405 nm) laser, e.g., MACSQuant® Analyzer 10 (# 130-096-343), MACSQuant X (# 130-105-100), or MACSQuant Analyzer 16 (# 130-109-803).
- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocol

2.1 Preparation of compensation controls

1. Determine cell number.
2. Prepare six tubes (VioGreen, VioBlue, PE, APC, FITC, blank), each with up to 5×10^5 MSCs.
3. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
4. Resuspend cells for VioGreen, VioBlue, PE, APC, and FITC compensation controls in 100 µL of buffer. Resupend cells for blank in 500 µL of buffer. This tube is ready-to-use.
5. Add 10 µL of CD73-Biotin into the tube for VioGreen, 10 µL of CD105-VioBlue into the tube for VioBlue, 10 µL of CD73-APC into the tube for APC, 10 µL of CD73-PE into the tube for PE, and 10 µL of CD90-FITC into the tube for FITC compensation controls.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (+2 to +8 °C).
7. Wash by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in 100 µL of buffer and add 10 µL of Biotin Antibody-VioGreen into the tube for VioGreen compensation control. Mix well and incubate for 10 minutes in the dark in the refrigerator (+2 to +8 °C).
9. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resupend each cell pellet separately in 500 µL of buffer.
11. Compensate instrument by following the instructions in the instrument user manual.

2.2 Immunofluorescent staining of cultured MSCs

▲ The following protocol is for working with cultured human MSCs. A protocol covering all steps from sample preparation and cell isolation to cell culture and flow cytometry is available at https://www.miltenyibiotec.com/applications/mesenchymal-stem-cells.html.

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

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 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 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Prepare two tubes (tube A, tube B) with up to 10^6 cells each.
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 MSC Phenotyping Cocktail, anti-human, REAfinity into tube A and 10 µL of MSC Control Cocktail, anti-human, REAfinity into tube B.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (+2 to +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.

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2.3 Flow cytometric data acquisition with the MACSQuant Analyzer 10

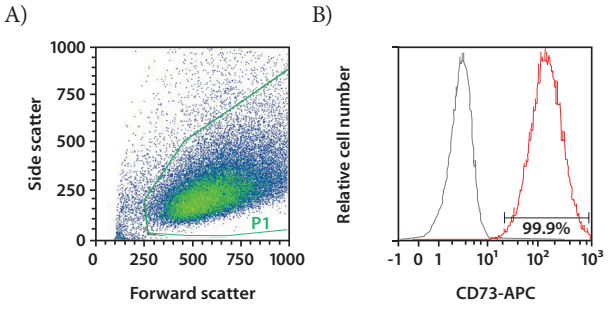
▲ Please refer to the MACSQuant Instrument user manual and software guide for detailed information on using the MACSQuant Analyzer.

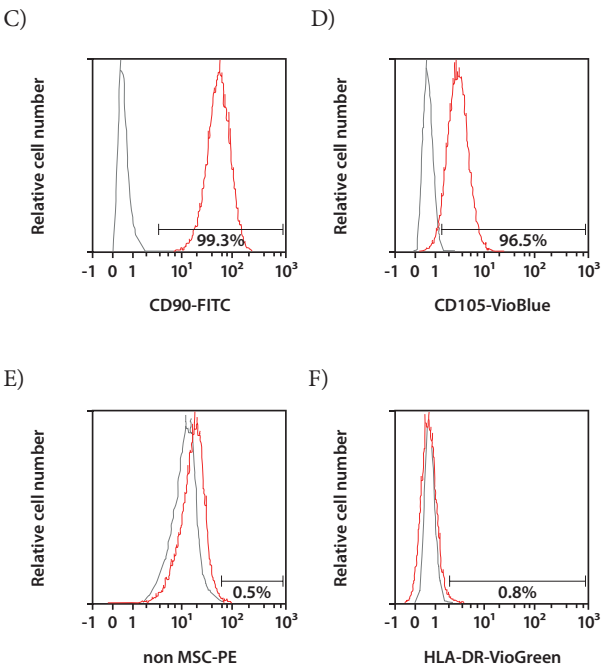
▲ The flow cytometer should be compensated for MSCs (refer to chapter 2.1).

1. Prepare and prime the MACSQuant Analyzer. Make sure the calibration and instrument settings of the instrument have been optimized for acquisition for the MSC Phenotyping Cocktail Kit, anti-human, REAfinity.
2. Analyze tube A (MSC Phenotyping Cocktail, anti-human, REAfinity) and tube B (MSC Control Cocktail, anti-human, REAfinity).
3. For doublets discrimination choose Height. Therefore, click the Advanced button located in the Channels tab and check Height.
4. Define an appropriate threshold based on forward scatter (FSC) versus side scatter (SSC) to exclude debris and from the data acquisition.
5. Start flow cytometric data acquisition.

3. Example of immunofluorescent staining with the MSC Phenotyping Cocktail Kit, anti-human, REAfinity

Cultured MSCs were stained with the MSC Phenotyping Cocktail Kit, anti-human, REAfinity and analyzed by flow cytometry using the MACSQuantAnalyzer 10.
As a preliminary step for exclusion of debris a gate in forward scatter area (FSC) versus side scatter (FSC) was set (A). Cells were analyzed for expression of CD73 (B), CD90 (C), CD105 (D), non-MSC markers (E), and HLA-DR (F). Each histogram was overlaid with the corresponding isotype control to identify positively stained cells. In order to distinguish between activated MSCs and non-MSCs, HLA-DR antibody conjugated to different fluorochromes has been used in combination with other MSC-negative markers (E, F).





Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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