

Contents

1. Description
 - 1.1 Background information
 - 1.2 Applications
 - 1.3 Reagent and instrument requirements
2. Protocol
 - 2.1 Preparation of compensation controls
 - 2.2 Immunofluorescent staining of cultured MSCs
 - 2.3 Flow cytometric data acquisition with the MACSQuant Analyzer 10
3. Example of immunofluorescent staining with the MSC Phenotyping Cocktail Kit, anti-human, REAfinity

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	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 5×10⁸ 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.

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, $\geq 95\%$ of the MSC population must express CD73, CD90, and CD105 and these cells must lack expression ($\leq 2\%$ positive) of CD34, CD45, CD11b or CD14, CD19 or CD79 α , and HLA-DR.

The MSC Phenotyping Cocktail Kit, anti-human, REAfinity 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 (2–8 °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 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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
4. Resuspend cells for VioGreen, VioBlue, PE, APC, and FITC compensation controls in 100 μ L of buffer. Resuspend cells for blank in 500 μ L of buffer. This tube is ready-to-use.
5. Add 10 μ L of CD73-Biotin into the tube for VioGreen, 10 μ L of CD105-VioBlue into the tube for VioBlue, 10 μ L of CD73-APC into the tube for APC, 10 μ L of CD73-PE into the tube for PE, and 10 μ L of CD90-FITC into the tube for FITC compensation controls.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
7. Wash by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
8. Add 10 μ L of Biotin Antibody-VioGreen into the tube for VioGreen compensation control. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
9. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
10. Resuspend each cell pellet separately in 500 μ L 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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
4. Resuspend each aliquot in 100 μ L of buffer.
5. Add 10 μ L of MSC Phenotyping Cocktail, anti-human, REAfinity into tube A and 10 μ L of MSC Control Cocktail, anti-human, REAfinity into tube B.

- 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.

- Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
- Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

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.

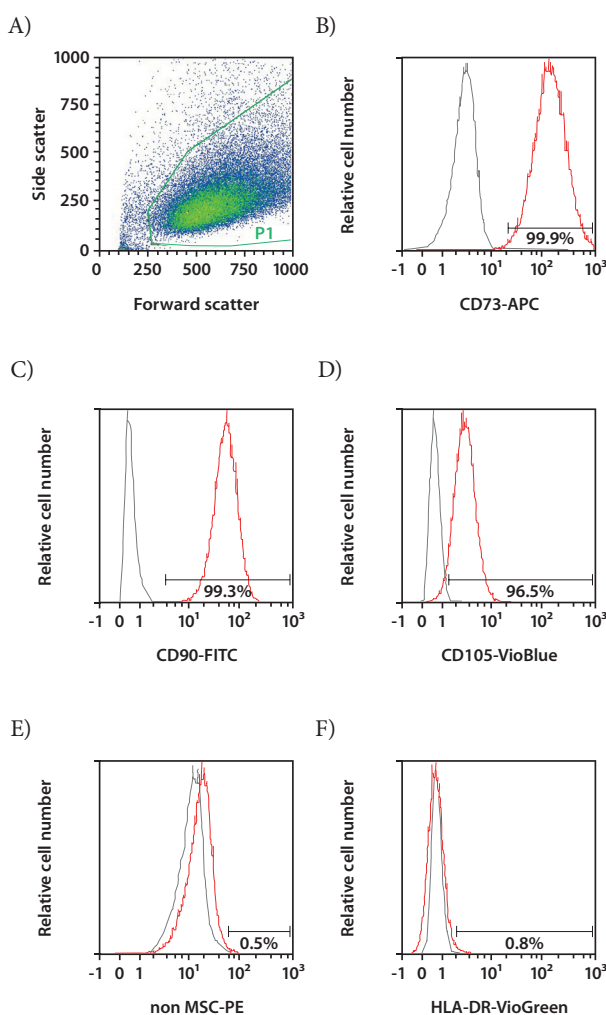
▲ The flow cytometer should be compensated for MSCs (refer to chapter 2.1).

- 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.
- Analyze tube A (MSC Phenotyping Cocktail, anti-human, REAfinity) and tube B (MSC Control Cocktail, anti-human, REAfinity).
- For doublets discrimination choose Height. Therefore, click the Advanced button located in the Channels tab and check Height.
- Define an appropriate threshold based on forward scatter (FSC) versus side scatter (SSC) to exclude debris and from the data acquisition.
- 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-MSCs (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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, MACS, MACSQuant, the Miltenyi Biotec logo, REAfinity, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.

Copyright © 2022 Miltenyi Biotec and/or its affiliates. All rights reserved.