Miltenyi Biotec

MSC Phenotyping Kit
human

Order no. 130-095-198

Contents

1. Description
   - 1.1 Background information
   - 1.2 Applications
   - 1.3 Recommended antibody dilution
   - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining and gating strategy with MSC Phenotyping Kit
4. Reference
5. Appendix

1. Description

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).

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 2×10^8 total cells.

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

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

1.1 Background information

Mesenchymal stem 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 *in vitro*. Cultivated MSCs should stain positive for CD73, CD90, and CD105 and should be negative for CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA-DR surface molecules.[1] The MSC Phenotyping Kit enables the fast, easy, and reliable phenotyping of cultured MSCs based on the defined ISCT standards.

1.2 Applications

- Identification and phenotyping of cultured MSCs based on the defined ISCT standards[1] by flow cytometry.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

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

a) The indicated antibody dilutions are for up to 10^6 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 Ca^{2+} or Mg^{2+} are not recommended for use.

140-002-819.02

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

- Anti-HLA-DR-VioBlue® (# 130-095-293) or Anti-HLA-DR-PerCP (# 130-095-291).
- (Optional) NH Expansion Medium, human (# 130-091-680) for expansion of nonhematopoietic (NH) stem cells.
- (Optional) NH Differentiation Media for differentiation of MSCs to adipocytes, chondrocytes, or osteoblasts with the NH AdipoDiff Medium, human (# 130-091-677), NH ChondroDiff Medium, human (# 130-091-679), or NH OsteoDiff Medium, human (# 130-091-678). For more information about media refer to www. miltenyibiotec.com.

2. General protocol for immunofluorescent staining

▲ The protocol is for working with cultured human MSCs. A detailed protocol for cultivating, differentiation, and detection of MSCs is including in the respective data sheet of the NH Expansion and NH Differentiation Media at www.miltenyibiotec.com/protocols.

▲ 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 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).

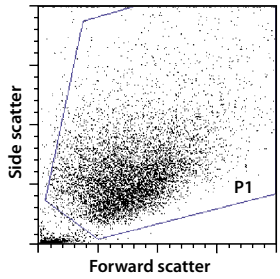
1. Determine cell number.
2. Take two aliquots (1 and 2), each with up to 1×10^6 cells.
3. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
4. Resuspend each aliquot in 100 µL of buffer.
5. Add 10 µL of the MSC Phenotyping Cocktail into aliquot 1 and 10 µL of the Isotype 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.

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:

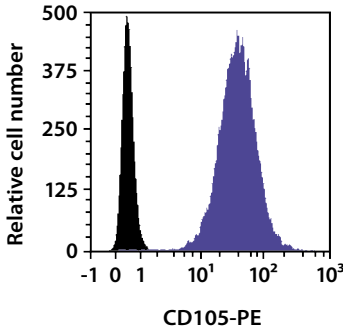
   B) FL-3 – analysis of the CD105-PE$^+$ cells

   C) FL-2 – analysis of the CD90-FITC$^+$ cells

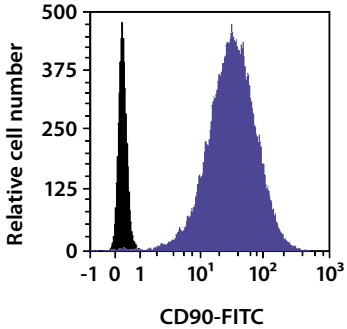
   D) FL-6 – analysis of the CD73-APC$^+$ cells

   E) FL-4 – analysis of the PerCP$^+$ cells

B)



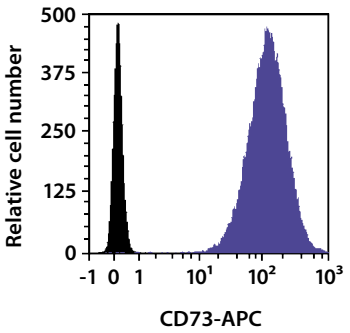
C)



140-002-819.02

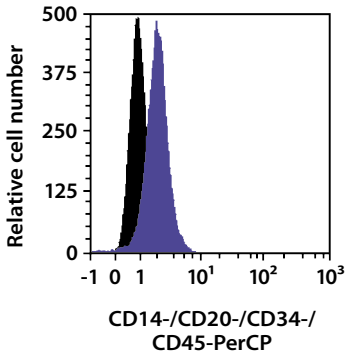
Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

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 cyometer

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×g for 10 minutes. Aspirate supernatant completely
4. Resuspend aliquots PerCP, PE, APC, and FITC in 100 µL of buffer, respectively.
5. Resupend aliqout blank in 500 µL of buffer. This aliquot is ready-to-use. Proceed directly with compensation.
6. Add 10 µL of CD73-Biotin in aliquot PerCP, CD105-PE in aliquot PE, 10 µL of CD73-APC in aliquot APC, and 10 µL 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×g for 10 minutes. Aspirate supernatant completely.
9. Add 10 µL 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×g for 10 minutes. Aspirate supernatant completely.
11. Resupend each cell pellet separately in 500 µL buffer.
12. Compensate instrument by following the instructions in the instrument user manual.

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

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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS, MACS, the MACS logo, MACSQuant, and VioBlue are registered trademarks of Miltenyi Biotec GmbH.

Copyright © 2011 Miltenyi Biotec GmbH. All rights reserved.

140-002-819.02

Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.