Miltenyi Biotec

Mo-DC Analysis Cocktail Kit, anti-human

Order no. 130-093-567

Contents

1. **Description**
   - **1.1 Background information**
   - **1.2 Applications**
   - **1.3 Reagent and instrument requirements**
2. **Protocol**
   - **2.1 Immunofluorescent staining of nucleated cells, e.g., PBMCs**
3. **Example of immunofluorescent staining with the Mo-DC Analysis Cocktail Kit, anti-human**

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	**1 mL Mo-DC Analysis Cocktail, anti-human** containing: CD14 Antibody, anti-human, FITC (clone: TÜK4, isotype: mouse IgG2a) CD83 Antibody, anti-human, APC (clone: HB15, isotype: mouse IgG1) CD209 (DC-SIGN) Antibody, anti-human, PE (clone: DCN47.5, isotype: mouse IgG1) **1 mL Mo-DC Control Cocktail, anti-human** containing: Isotype Control Antibody, mouse IgG2a, FITC (clone: S43.10) Isotype Control Antibody, mouse IgG1, PE (clone: IS5-21F5) Isotype Control Antibody, mouse IgG1, APC (clone: IS5-21F5)
Capacity	100 tests or up to 10^9 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

Blood monocytes derive from monoblasts in the bone marrow and have the capacity to differentiate into macrophages and monocyte-derived dendritic cells (Mo-DCs). Since the frequency of DCs in peripheral blood is limited, the *in vitro* generation of Mo-DCs is widely used to gain access to larger cell numbers of a homogenous, well-defined DC population. The Mo-DC Analysis Cocktail Kit, anti-human is designed to enable the easy and rapid evaluation of the *in vitro* generation of Mo-DCs. Its use minimizes pipetting steps due to the antibody premixture; all staining antibodies are optimally titrated and simply added to an aliquot of the separated or differentiated monocytes in a single step before flow cytometry. The Mo-DC Analysis Cocktail Kit, anti-human comprises monoclonal antibodies recognizing CD14, CD83, and CD209 and corresponding isotype controls.
CD14 is strongly expressed on human monocytes. CD83 is a member of the immunoglobulin superfamily with a single V-type extracellular domain. Beside CD80 and CD86, CD83 is regarded as a maturation marker for DCs. CD209 (DC-SIGN) belongs to the type II c-type lectin superfamily and binds various ligands including glycoprotein gp-120 of human immunodeficiency virus, hepatitis C virus, Dengue virus and mycobacterium tuberculosis. CD209 appears to regulate DC and T cell interactions, facilitates DC migration via ICAM-2, and interacts with ICAM-3 on resting T cells. CD209 expression is limited to tissue DCs and Mo-DCs.

1.2 Applications

- Evaluation of the differentiation stage of Mo-DCs.

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 Ca^{2+} or Mg^{2+} are not recommended for use.

- Flow cytometer equipped with a red (640 nm) and a blue (488 nm) laser, e.g., MACSQuant® Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells without fixation.
- (Optional) CD14 MicroBeads, human (# 130-050-201), Classical Monocyte Isolation Kit, human (# 130-117-337), or Pan Monocyte Isolation Kit, human (# 130-096-537) for separation of monocytes from peripheral blood mononuclear cells (PBMCs).

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-002-333.05

- (Optional) MACS Comp Bead Kit, anti-mouse Igκ (# 130-097-900) or anti-human Igκ (# 130-104-187) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocol

2.1 Immunofluorescent staining of nucleated cells, e.g., PBMCs

▲ Prepare two samples of the original fraction, one sample for staining with the Mo-DC Analysis Cocktail, anti-human and one sample for staining with the Mo-DC Control Cocktail, anti-human.

▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 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 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

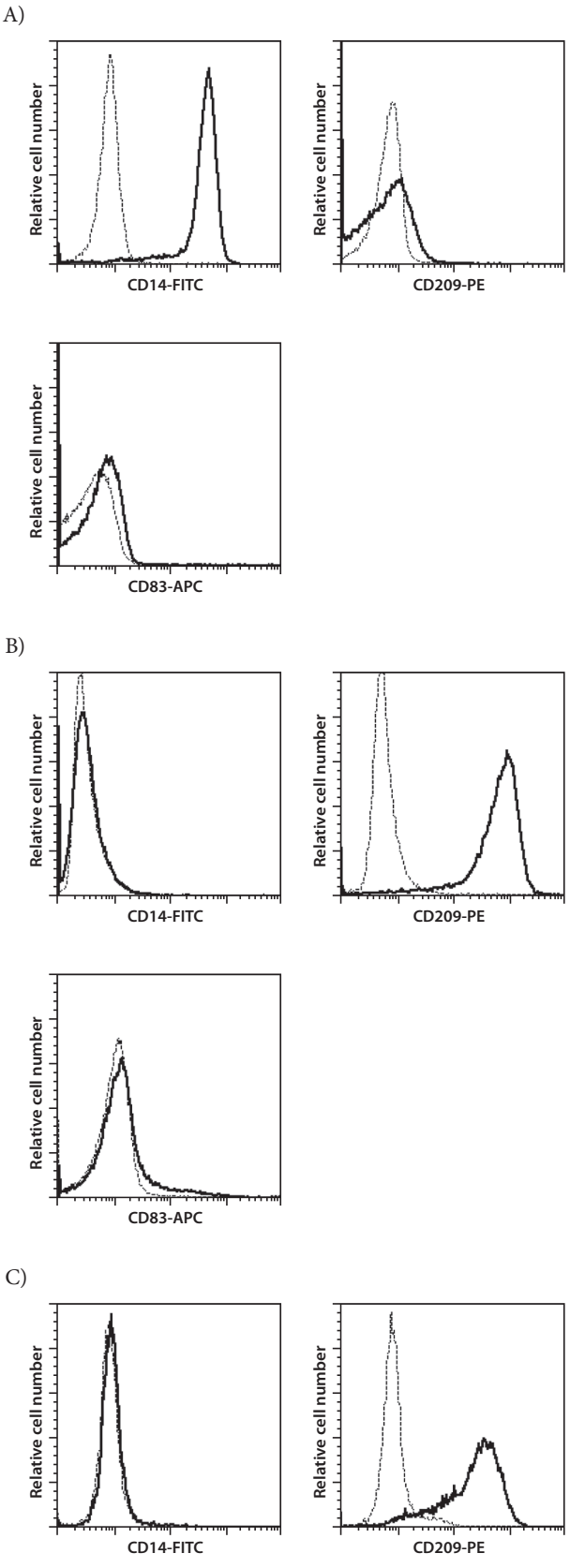
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Mo-DC Analysis Cocktail, anti-human to one sample and 10 µL of Mo-DC Control Cocktail, anti-human to the other sample.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

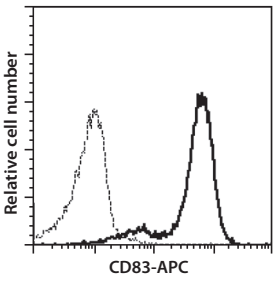
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

   ▲ **Note:** Store samples at 2–8 °C protected from light until analysis.
8. (Optional) Add Propidium Iodide Solution to each tube directly before data acquisition according to the concentration stated in the respective data sheet.

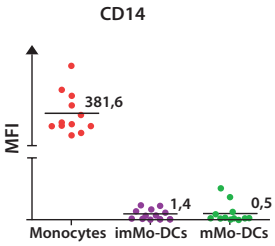
3. Example of immunofluorescent staining with the Mo-DC Analysis Cocktail Kit, anti-human

Flow cytometric analysis of *in vitro* generated Mo-DCs was performed using the Mo-DC Analysis Cocktail Kit, anti-human. Monocytes isolated with CD14 MicroBeads (# 130-050-201) from PBMCs were differentiated into Mo-DCs according to standard protocols. In brief, immature Mo-DCs were generated by culturing purified $CD14^+$ monocytes (10^6/mL) in the presence of GM-CSF (1000 U/mL) and IL-4 (400 U/mL) for 7 days. Cytokines were refreshed on day 3 by adding the same volume of starting medium. On day 7 cells were washed and further cultured for 3 days in the presence of TNF-alpha (100 ng/mL). Flow cytometric analysis of CD14, CD209, and CD83 expression was performed on day 0, 7, and 10. Dashed lines show corresponding isotype control stainings. Analyses were performed of monocytes (A), immature Mo-DCs (B), and mature Mo-DCs (C). Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. Expression levels were calculated as mean fluorescence intensities (MFIs) for CD14 (D), CD209 (E), and CD83 (F) for monocytes, immature Mo-DCs (imMo-DCs), and mature Mo-DCs (mMo-DCs) from 12 donors.

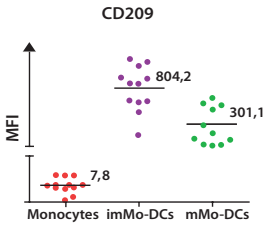




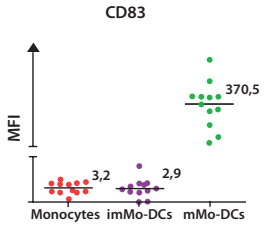
D)



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, and the Miltenyi Biotec logo 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.