MDSC Phenotyping Cocktail Kit, anti-human, REAfinity™

Order no. 130-126-233

Contents

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 MDSC Phenotyping Cocktail, anti-human, REAfinity:** CD11b Antibody, anti-human, VioBlue®, REAfinity (clone: REA713, isotype: recombinant human IgG1) CD45 Antibody, anti-human, VioGreen™, REAfinity (clone: REA747, isotype: recombinant human IgG1) HLA-DR Antibody, anti-human, FITC, REAfinity (clone: REA805, isotype: recombinant human IgG1) CD33 Antibody, anti-human, PE, REAfinity (clone: REA775, isotype: recombinant human IgG1) CD16 Antibody, anti-human, PE-Vio® 770, REAfinity (clone: REA423, isotype: recombinant human IgG1) CD14 Antibody, anti-human, APC-Vio 770, REAfinity (clone: REA599, isotype: recombinant human IgG1) 7-AAD Staining Solution **1 mL MDSC Control Cocktail, anti-human, REAfinity:** CD11b Antibody, anti-human, VioBlue, REAfinity (clone: REA713, isotype: recombinant human IgG1) CD45 Antibody, anti-human, VioGreen, REAfinity (clone: REA747, isotype: recombinant human IgG1) REA Control (S) Antibody, human IgG1, FITC, REAfinity (clone: REA293) REA Control (S) Antibody, human IgG1, PE, REAfinity (clone: REA293) CD16 Antibody, anti-human, PE-Vio 770, REAfinity (clone: REA423, isotype: recombinant human IgG1) CD14 Antibody, anti-human, APC-Vio 770, REAfinity (clone: REA599, isotype: recombinant human IgG1) 7-AAD Staining Solution **0.1 mL CD16 Antibody, anti-human, PE-Vio 770, REAfinity** (clone: REA423, isotype: recombinant human IgG1) **0.1 mL CD14 Antibody, anti-human, APC-Vio 770, REAfinity** (clone: REA599, isotype: recombinant human IgG1)
Capacity	50 tests or 5×10^8 total cells or 10 mL whole blood.
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

Myeloid-derived suppressor cells (MDSCs) are a heterogeneous group of mononuclear and polymorphonuclear myeloid cells with different immunosuppressive as well as disease promoting functions. In healthy humans, they are found in very small numbers, but can expand considerably in pathological conditions. Depending on the type and stage of the disease, the MDSC population might be present in different maturation phases. In humans, three major MDSC subsets can be identified: low density polymorphonuclear-MDSCs (PMN-MDSCs), also known as granulocytic MDSCs (G-MDSCs), monocytic-MDSCs (M-MDSCs), and early-MDSCs (e-MDSCs). Validated unambiguous phenotypic markers for MDSCs are still lacking and therefore various combinations of specificities are used for their identification.

The MDSC Phenotyping Cocktail Kit, anti-human, REAfinity is intended to be used for standardization and simplification of flow cytometric analysis of the MDSC subpopulations described so far. For greater flexibility, the product configuration also allows

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

140-005-758.02

the extension of the analysis panel with additional specificities of interest (e.g. CD10, CD66b).
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

- Detection and quantification of MDSC subsets in human PBMCs and other starting materials, e.g., whole blood.

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 at least a red (640 nm), a blue (488 nm), and a violet (405 nm) laser e.g., MACSQuant® Analyzer 10 (# 130-096-343).
- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183) for lysis of red blood cells for analysis of MDSC subsets in whole blood.
- (Optional) Additional APC-conjugated antibody of choice, for example, CD66b-APC, human (clone: REA306), CD15-APC, human (clone: VIMC6), LOX-1 Antibody-APC, human (clone: REA1188), CD10-APC, human (clone: REA877), REA Control (S) Antibody-APC. For more information on antibodies refer to www.miltenyibiotec.com/antibodies.

2. Protocol

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

▲ Volumes given below are optimized for staining of **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. For each biological sample, prepare two tubes containing up to 10^7 nucleated cells each.
3. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
4. Resuspend up to 10^7 nucleated cells per 200 µL of buffer.
5. Add 20 µL of MDSC Phenotyping Cocktail, anti-human, REAfinity to the first tube.
6. Add 20 µL of MDSC Control Cocktail, anti-human, REAfinity to the second tube.
7. (Optional) In case of analysis based on additional markers for pre-gating (e.g. CD66b or CD15), add 4 µL of the optional APC-conjugated antibody to each of both tubes.
   For a more detailed phenotypical analysis of PMN-MDSC subsets (e.g. LOX-1, CD10), add 4 µL of APC-conjugated antibody to the tube containing the MDSC Phenotyping Cocktail, anti-human, REAfinity and 4 µL of REA Control (S) Antibody-APC to the tube containing the MDSC Control Cocktail, anti-human, REAfinity.
8. 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.
9. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. 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.
11. Proceed to flow cytometric analysis.

2.2 Immunofluorescent staining and lysis of whole blood (lyse/wash)

▲ When starting from whole blood a lysis step is required. It is recommended to use Red Blood Cell Lysis Solution (10×) (# 130-094-183).

▲ In order to distinguish high-density PMN from low density PMN-MDSCs, a gradient centrifugation prior to staining is required. For analysis of whole blood or lysed whole blood samples, use additional markers of choice to distinguish the above mentioned populations.

1. Dilute Red Blood Cell Lysis Solution (10×) 1:10 with double-distilled water (ddH_2O), for example, dilute 1 mL of Red Blood Cell Lysis Solution (10×) with 9 mL of ddH_2O.

   ▲ **Note:** Do not dilute with deionized water. Store prepared 1× Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.
2. For each sample, prepare two tubes containing 200 µL of whole blood each.
3. Add 20 µL of MDSC Phenotyping Cocktail, anti-human, REAfinity to the first tube.
4. Add 20 µL of MDSC Control Cocktail, anti-human, REAfinity to the second tube.
5. (Optional) In case of analysis based on additional markers for pre-gating (e.g. CD66b or CD15), add 4 µL of the optional APC-conjugated antibody to each of both tubes.
   For a more detailed phenotypical analysis of PMN-MDSC subsets (e.g. LOX-1, CD10), add 4 µL of APC-conjugated antibody to the tube containing the MDSC Phenotyping Cocktail, anti-human, REAfinity and 4 µL of REA Control (S) Antibody-APC to the tube containing the MDSC Control Cocktail, anti-human, REAfinity.

140-005-758.02

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. Add 2 mL of 1× Red Blood Cell Lysis Solution and immediately vortex thoroughly for 5 seconds. Incubate for 10–15 minutes in the dark at room temperature.

8. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

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

10. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

11. Proceed immediately to flow cytometric analysis.

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

All results shown in this example were obtained from a selected healthy blood donor who had an elevated number of MDSCs. PBMCs were prepared from fresh blood samples. Gradient centrifugation was performed less than 30 minutes after blood collection. Cells were stained using the MDSC Phenotyping Cocktail Kit, anti-human, REAfinity. Cells were analyzed by flow cytometry using the MACSQuant Analyzer 10.

A) To exclude red blood cells and identify leukocytes, a first gate on $CD45^+$ cells was set (region R1). To eliminate doublets, a gate on single cells in forward scatter-area (FSC-A) versus forward scatter-height (FSC-H) was set (region R2). These cells were further distinguished from debris via FSC-A and side scatter-are (SSC-A) (region R3). A gate on viable cells ($7\text{-}AAD^-$ cells) was set (region R4).

B) Cells from region R4 were further separated into three subsets: SSC^{high} cells, which correspond to low density PMN-MDSCs (region R5), $CD14^+$ cells (region R6), and $SSC^{low}CD14^-$ cells (region R7). Low density PMN-MDSCs contained in region R5 were further separated into four subsets based on the expression of CD16 and CD11b (regions R8–R11).

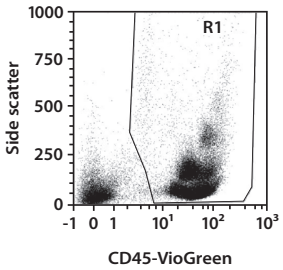
C) Cells stained with MDSC Control Cocktail, anti-human, REAfinity from R6 were displayed with CD14 versus REA Control (S) Antibody-FITC and a region enclosing >99.5% of cells was set (region R12). This region was transferred to the cells labeled with MDSC Phenotping Cocktail, human depicting M-MDSCs as $CD14^+HLA\text{-}DR^-$ cells.

D) Cells stained with MDSC Control Cocktail, anti-human, REAfinity from region R7 were displayed with REA Control (S) Antibody-FITC versus REA Control (S) Antibody-PE and a region above the background staining containing <0.5% of cells was drawn (region R13). This region was transferred to the cells labeled with MDSC Phenotyping Cocktail, anti-human, REAfinity depicting e-MDSCs as $HLA\text{-}DR^-CD33^{int}$ cells.

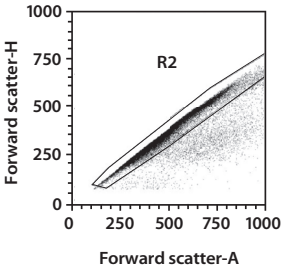
For an example of staining with optional markers, refer to the application protocol "Identification of human myeloid-derived suppressor cell (MDSC) subpopulations" at www.miltenyibiotec.com/applications.

A) Gating strategy

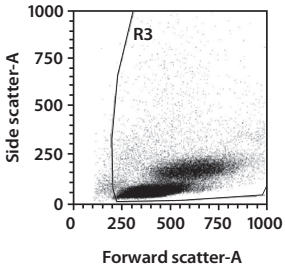
All events



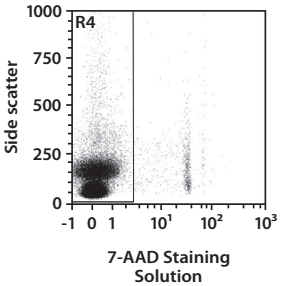
Gated on R1



Gated on R2

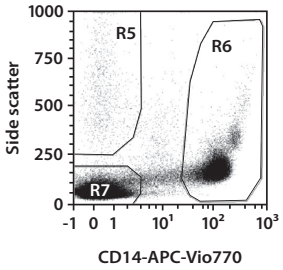


Gated on R3

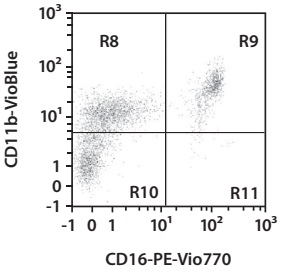


B) Identification of low density PMN cells (PMN-MDSCs)

Gated on R4

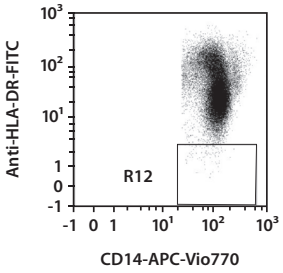


Gated on R5

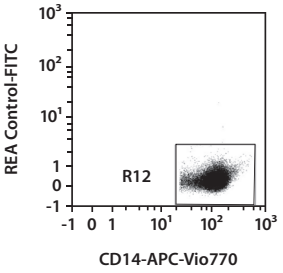


C) Identification of M-MDSCs

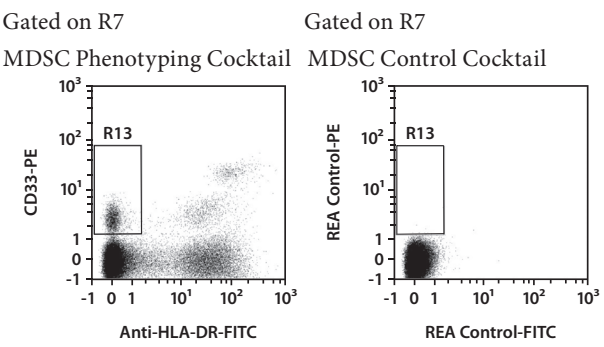
Gated on R6
MDSC Phenotyping Cocktail



Gated on R6
MDSC Control Cocktail



D) Identification of e-MDSCs



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, Vio, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.