Miltenyi Biotec

Treg Surface Marker Analysis Cocktail, anti-human

250 µL	130-096-076
1 mL	130-096-082

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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	**250 µL Treg Surface Marker Analysis Cocktail, anti-human** or **1 mL Treg Surface Marker Analysis Cocktail, anti-human** containing: CD4 Antibody, anti-human, FITC (clone: VIT4, isotype: mouse IgG2a) CD25 Antibody, anti-human, APC (clone: 4E3, isotype: mouse IgG2b) CD45 Antibody, anti-human, VioBlue® (clone: 5B1, isotype: mouse IgG2a) CD127 Antibody, anti-human, PE (clone: MB15-18C9, isotype: mouse IgG2a)
Capacity	25 tests or up to 2.5×10^8 total cells or 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

The Treg Surface Marker Analysis Cocktail, anti-human has been developed as a simple one-reagent-solution for identification of regulatory T cells (Tregs). It combines the standard Treg markers CD4, CD25, and CD127 as well as the leukocyte marker CD45 at pre-optimized titers in one vial. The Treg Surface Marker Analysis Cocktail, anti-human simplifies the flow cytometric evaluation of Tregs separated using MACS Technology. The cocktail is optimized for staining or separation control using the MACSQuant Analyzer, but can also be used on other flow cytometers. The CD45-VioBlue antibody requires the use of a violet laser for flow cytometric detection (405 nm).

Treg cells are a subset of T cells with the ability to suppress harmful immunological reactions to self and foreign antigens. The combination of staining for CD4, CD25, and CD127 is the current standard method of identification of live Treg cells. Several publications have reported a strong correlation between the $CD25^+CD127^{dim/neg}$ phenotype and expression of FoxP3 and suppressive function in $CD4^+$ cells. The Treg cell marker FoxP3 can only be stained on fixed and permeabilzed cells which are no longer viable for culture or downstream applications.

1.2 Applications

- Staining and enumeration of $CD4^+CD25^+CD127^{dim/neg}$ regulatory T cells in preparations of peripheral blood mononuclear cells (PBMCs), apheresis, umbilical cord blood, or whole blood.
- Analysis of Treg cells separated using MACS Technology, e.g., human $CD4^+CD25^+$ Treg cells can be isolated using the $CD4^+CD25^+$ Regulatory T Cell Isolation Kit, human (# 130-091-301).

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), a blue (488 nm), and a violet (405 nm) laser, e.g., MACSQuant® Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803).
- (Optional) MACS Comp Bead Kit, anti-mouse Igκ (# 130-097-900) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells without fixation.

140-003-179.03

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- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183) for staining of regulatory T cells in whole blood samples.
- (Optional) Double-distilled water (ddH_2O).

2. Protocol

▲ The Treg Surface Marker Analysis Cocktail, anti-mouse should be used at a dilution of 1:11 for up to 10^7 cells in 100 µL.

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

▲ 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 Treg Surface Marker Analysis Cocktail, anti-human.
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) To exclude dead cells and debris from the analysis, add Propidium Iodide Solution to each tube directly before data acquisition.
9. Proceed to flow cytometric analysis.

2.2 Immunofluorescent staining and lysis of whole blood (lyse/no wash)

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double-distilled water (ddH_2O), for example, dilute 1 mL of 10× Red Blood Cell Lysis Solution 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. Add 10 µL Treg Surface Marker Analysis Cocktail, anti-human per 100 µL of whole blood.
3. Mix well and incubate for 20 minutes in the dark at room temperature.

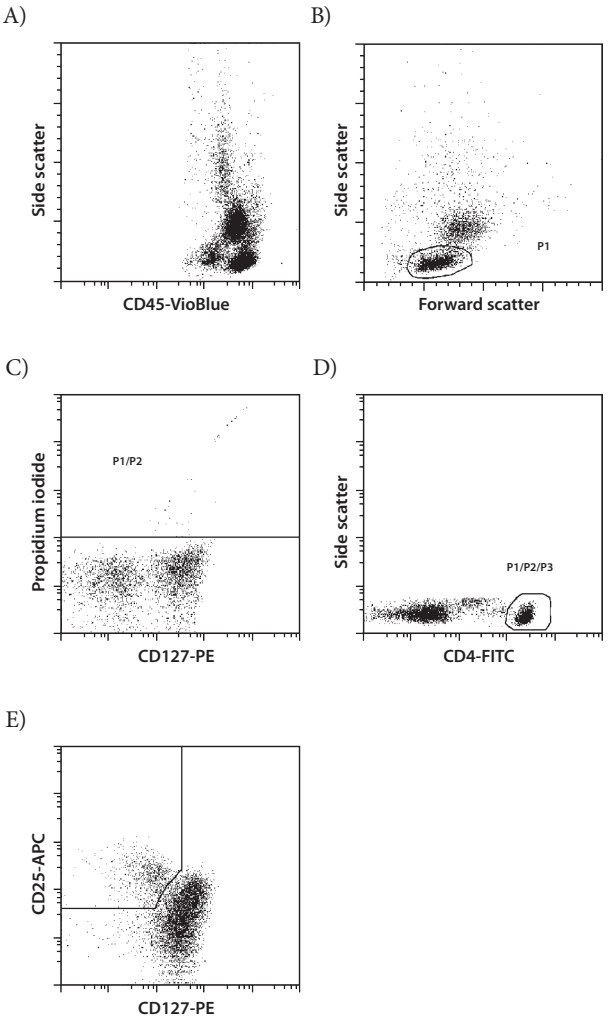
   ▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
4. Add 1 mL of 1× Red Blood Cell Lysis Solution and immediately vortex thoroughly for 3 seconds. Incubate for 10 minutes in the dark at room temperature.

   ▲ **Note:** If the sample is not translucent red after 10 minutes, incubate for another 2 minutes.
5. (Optional) Add Propidium Iodide Solution to each tube directly before data acquisition.
6. Proceed immediately to flow cytometric analysis.

   ▲ **Note:** Store samples at 2–8 °C protected from light until analysis.

3. Example of immunofluorescent staining with the Treg Surface Marker Analysis Cocktail, anti-human

Whole blood from a healthy donor was stained with the Treg Surface Marker Analysis Cocktail, anti-human using the protocol for staining samples from whole blood (lyse/no wash). Cells were analyzed by flow cytometry using the MACSQuant Analyzer 10.
To identify the major circulating blood cell types CD45 was used to target all leukocytes (A). As a step for elimination of debris, a gate in forward scatter area (FSC-A) versus side scatter area (SSC-A) was set (data not shown). Theses cells were further gated for lymphocytes (B). Dead cells were excluded by gating on propidium iodide–negative cells (C). $CD4^+$ cells were defined (D) and analyzed for expression of CD25 and CD127 (E).



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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