Miltenyi Biotec

Treg Phenotyping Kit, anti-human, REAfinity™

Order no. 130-122-994

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

200 µL CD45 Antibody, anti-human, VioBlue®, REAfinity (clone: REA747, isotype: recombinant human IgG1)

200 µL CD4 Antibody, anti-human, VioGreen™, REAfinity (clone: REA623, isotype: recombinant human IgG1)

200 µL CD25 Antibody, anti-human, Vio® Bright B515, REAfinity (clone: REA570, isotype: recombinant human IgG1)

200 µL CD127 Antibody, anti-human, PE, REAfinity (clone: REA614, isotype: recombinant human IgG1)

200 µL FoxP3 Antibody, anti-human, Vio R667, REAfinity (clone: REA944, isotype: recombinant human IgG1)

25 mL Fixation/Permeabilization Solution 1

2× 40 mL Fixation/Permeabilization Solution 2

40 mL Permeabilization Buffer (10×)

1 mL of Tandem Signal Enhancer, human

Capacity

100 tests or up to 10^8 total cells.

Product format

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

Storage

Store Permeabilization Buffer (10×) at room temperature. Store all other reagents 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 Phenotyping Kit, anti-human, REAfinity has been developed for the convenient detection of $CD45^+CD4^+CD25^+CD127^{dim/neg}FoxP3^+$ regulatory T cells (Tregs) by flow cytometry. Tregs are a subset of T cells with the ability to suppress harmful immunological reactions to self and foreign antigens. Due to the lack of a specific marker for Tregs, a combination of CD4, CD25, CD127, and FoxP3 is commonly used for their identification.

The Treg Phenotyping Kit, anti-human, REAfinity includes all reagents necessary for cell surface staining of CD4, CD25, and CD127 as well as the leukocyte marker CD45 and intracellular staining of FoxP3 together with a special staining buffer set and an optimized protocol.

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 enumeration of $CD45^+CD4^+CD25^+CD127^{dim/neg}FoxP3^+$ regulatory T cells by flow cytometry.
- Analysis of Treg cells separated using MACS Technology by flow cytometry. 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).
- Deionized or distilled water.

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140-005-561.04

- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

2. Protocol

▲ For a detailed gating strategy refer to the application protocol "Immunophenotyping of regulatory T cells from human PBMCs using flow cytometry" at www.miltenyibiotec.com/applications.

2.1 Reagent preparation

▲ Always prepare reagents freshly. Failure to do so may lead to suboptimal results.

▲ The required total buffer volumes should be calculated beforehand; volumes will depend on the number of cells to be analyzed as well as the number of tests to be performed.

Fixation/Permeabilization Solution
To achieve the appropriate working concentration for safe fixation and permeabilization of cells, the Fixation/Permeabilization Solution 1 must be diluted 1:4 with the Fixation/Permeabilization Solution 2 (i.e. for 10^6 cells use 0.25 mL of Fixation/Permeabilization Solution 1 plus 0.75 mL of Fixation/Permeabilization Solution 2).

Permeabilization Buffer
To achieve the appropriate working concentration for safe permabilization of cells, the 10× Permeabilization Buffer must be diluted 1:10 with deionized or distilled water before use (i.e. 1 mL of 10× Permeabilization Buffer plus 9 mL of deionized or distilled water).

▲ **Note:** Before preparing the dilution make sure that buffer does not contain any precipitates.

2.2 Immunofluorescent staining of Tregs

▲ 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 except for the fixation step due to the impact on cell morphology. 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).

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

2.2.1 Surface staining of CD45, CD4, CD25, and CD127

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 92 µL of buffer.
4. Add 2 µL of each of CD45-VioBlue, CD4-VioGreen, CD25-Vio Bright B515, and CD127-PE.
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 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
7. Proceed immediately to 2.2.2.

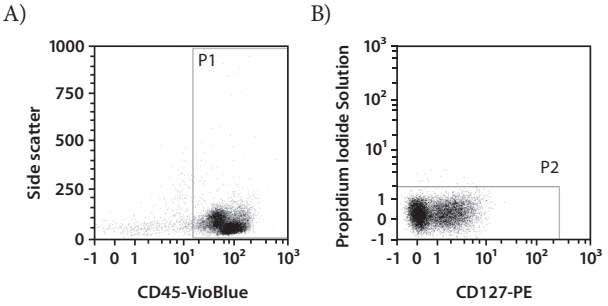
2.2.2 Intracellular staining of FoxP3

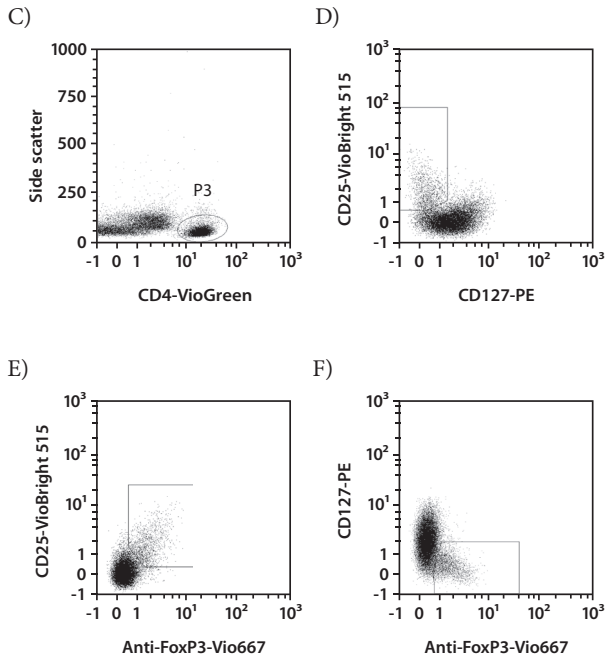
1. Resuspend 10^6 nucleated cells in 1 mL of cold, freshly prepared Fixation/Permeabilization Solution.
2. Mix well and incubate for 30 minutes in the dark in the refrigerator (2–8 °C).
3. Wash cells by adding 1–2 mL of cold buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
4. Wash cells by adding 1–2 mL of cold 1× Permeabilization Buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
5. Resuspend up to 10^6 nucleated cells in 10 µL of Tandem Signal Enhancer, human and 88 µL of cold 1× Permeabilization Buffer.
6. Add 2 µL of FoxP3 Antibody-Vio R667.
7. Mix well and incubate for 30 minutes in the dark in the refrigerator (2–8 °C).
8. Wash cells by adding 1–2 mL of cold 1× Permeabilization Buffer per 10^6 cells and centrifuge at 300×g for 5 minutes at 4 °C. Aspirate supernatant completely.
9. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

   ▲ **Note:** Due to fixation and permeabilization, cells can be smaller than viable cells. Thus, FSC/SSC settings of the flow cytometer might need to be adjusted.

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

Human peripheral blood mononuclear cells (PBMCs) were stained with CD45-VioBlue, CD4-VioGreen, CD25-Vio Bright B515, and CD127-PE antibodies. Cells were fixed, permeabilized, and stained with FoxP3 Antibody-Vio R667. Cells were analyzed by flow cytometry. Lymphocytes expressing CD45 were gated as P1 (A). From the P1-gated population, autofluorescent cell debris was excluded as P2 (B). From P2, CD4-expressing cells were gated as P3 (C). From the P3-gated population, Treg cells were shown using different gates that compare CD25, CD127, and FoxP3 expression (D–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.

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