Anti-IL-10 antibodies, human

For research use only

6
One test corresponds to labeling of up to 10 cells in a total volume of 100 µL

Product	Content	Order no.
Anti-IL-10-PE	for 100 tests	130-112-728
Anti-IL-10-PE	for 30 tests	130-112-917
Anti-IL-10-APC	for 30 tests	130-112-918
Anti-IL-10-APC	for 100 tests	130-112-729

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.

Technical data and background information

Antigen	IL-10
Clone	REA842
Isotype	recombinant human IgG1
Isotype control	REA Control (I) antibodies
Alternative names of antigen	IL10, CSIF, GVHDS, TGIF, IL10A, Interleukin-10
Entrez Gene ID	3586
Molecular mass of antigen [kDa]	19
Cross-reactivity	rhesus monkey (*Macaca mulatta*)
Distribution of antigen	T cells, monocytes, macrophages, dendritic cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

Clone REA842 recognizes the interleukin 10 (IL-10). IL-10 is a cytokine predominantly secreted by $CD4^+$ memory and effector T cells and antigen-presenting cells, for example, monocytes/macrophages and dendritic cells. IL-10 has important suppressive functions on immune responses and is believed to be involved in the maintenance of tolerance. IL-10 blocks activation of cytokine synthesis by T_H1 cells, activated monocytes, and NK cells. It can stimulate immunoglobulin production by B cells. Additional information: Clone REA842 displays negligible binding to Fc receptors.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells containing Inside Fix and Inside Perm.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

Protocol for intracellular staining of cells

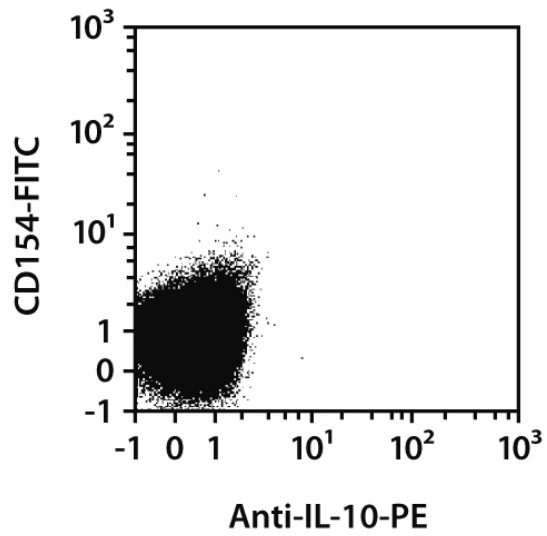
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

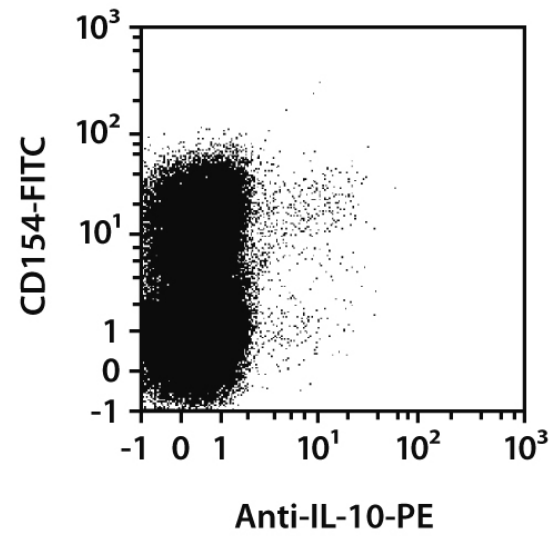
1. Wash up to 10^6 cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. (Optional) Stain cell surface antigens that are sensitive to fixation with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 cells in 250 µL of buffer.
4. Add 250 µL of Inside Fix (Inside Stain Kit). Mix well and incubate for 20 minutes in the dark at room temperature.
5. Centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
   Note: Fixed cells may be stored in azide-containing buffer at 2–8 °C for up to 1 week.
7. (Optional) Stain cell surface antigens that are sensitive to permeabilization with appropriate antibodies according to the manufacturer's recommendations. Then wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Wash cells by adding 1 mL of Inside Perm (Inside Stain Kit) and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
9. Resuspend cells in 98 µL of Inside Perm. Add 2 µL of the antibody.
   Note: For staining with several antibodies in this step, reduce the volume of Inside Perm accordingly. For efficient permeabilization, the volume of Inside Perm should be at least 30% of the overall staining volume.
10. Mix well and incubate for 10 minutes in the dark at room temperature.
11. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
12. (Optional) If biotinylated antibody was used, resuspend the cell pellet in Inside Perm and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
13. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy. Store cells at 2–8 °C in the dark until analysis. Mix well before flow cytometric acquisition.

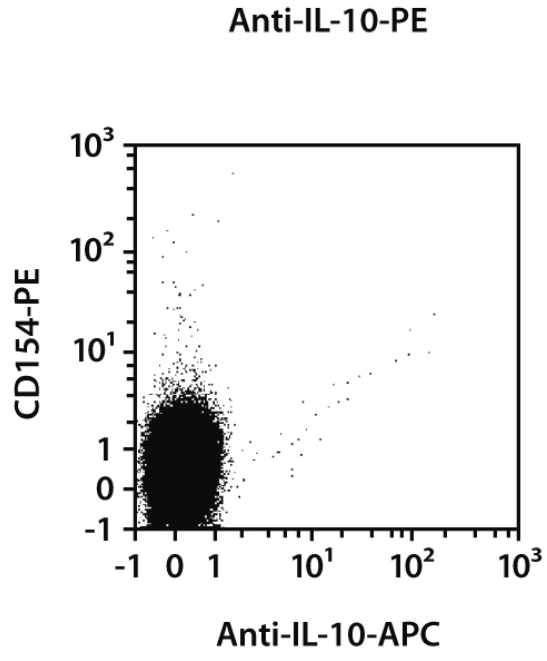
- Note: Samples may be stored at 2–8 °C in the dark for up to 24 hours.
- Note: Do not use propidium iodide (PI) or 7-AAD staining.

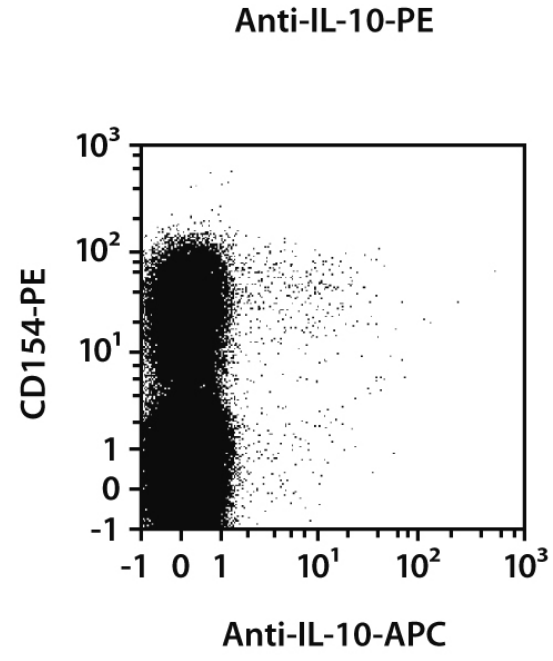
Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs) were either left unstimulated (left image) or stimulated with 50 ng/mL PMA and 1 µg/mL ionomycin for 6 hours. After two hours 1 µg/mL brefeldin A was added. Cells were then fixed and permeabilized followed by intracellular staining with Anti-IL-10 antibodies as well as with CD154 antibodies. Flow cytometry was performed using the MACSQuant®Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals.









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