Anti-IL-4 antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 µL.

Product	Content	Order no.
Anti-IL-4-PE	for 30 tests	130-098-815
Anti-IL-4-PE	for 100 tests	130-091-647
Anti-IL-4-PE-Vio615	for 30 tests	130-107-199
Anti-IL-4-PE-Vio615	for 100 tests	130-107-144

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-4
Clone	7A3-3
Isotype	mouse IgG1κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	BCGF-1, BCGF1, BSF-1, BSF1
Molecular mass of antigen [kDa]	15
Cross-reactivity	rhesus monkey (*Macaca mulatta*), cynomolgus monkey (*Macaca fascicularis*)
Product format	Antibodies 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.

Interleukin-4 (IL-4) is predominantly secreted by activated $CD4^+$ memory and effector T_H2 cells, basophils, and mast cells. It induces and supports humoral immune responses for the neutralization of extracellular pathogens. The T_H2 types of immune mechanisms can also be involved in immunological disorders such as IgE-mediated allergy.
The Anti-IL-4 antibody has been designed for intracellular staining of IL-4–producing cells. Cells can be stimulated for IL-4–production, e.g., by polyclonal stimulation with mitogens. For induction of IL-4 production by antigen-specific T cells, cells are restimulated with respective antigen. IL-4 can be accumulated in the cells by addition of secretion inhibitors like brefeldin A. After fixation and permeabilization of the cell sample, IL-4–producing cells can be stained intracellularly with the Anti-IL-4 antibody. Staining of surface markers allows simultaneous flow cytometric analysis of subsets and activation status of the IL-4–producing cells. Magnetically enriched cells can be stained intracellularly

for IL-4 production directly on the MACS® Column. This procedure ensures higher sensitivity of detection and minimizes loss of cells during washing procedures for cytokine analysis of rare cells, e.g., $CD4^+$ T cells in HIV patients, or other cell sources than peripheral blood mononuclear cells (PBMCs), e.g., bronchoalveolar lavages, or synovial fluids.

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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (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 1:10 for up to 10^7 cells/100 µL of buffer.
- It is recommended to stain 10^6 cells per sample. When working with up to 10^7 cells, use 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).
- The special protocol “Intracellular staining in combination with magnetic cell separation” is available for download at www.miltenyibiotec.com/protocols. In-column intracellular staining of cells immobilized on an MS Column is especially advantageous for the analysis of rare cells.

1. Wash up to 10^7 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 10^7 cells in 500 µL of buffer.
4. Add 500 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 90 µL of Inside Perm. Add 10 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 100 µL of Inside Perm,

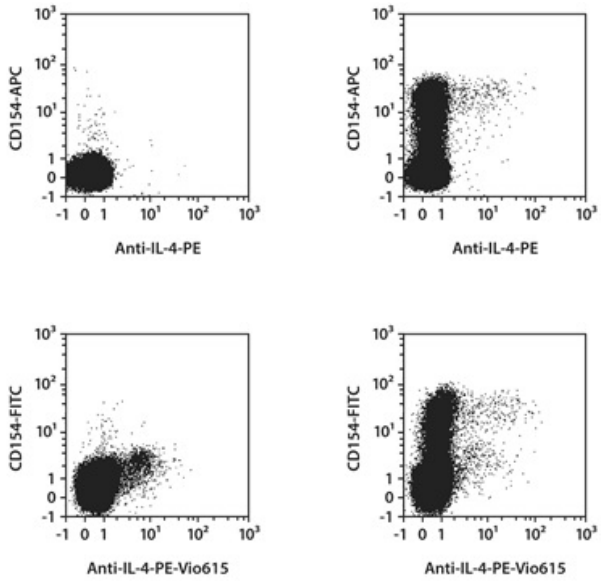
add 10 µL of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 10 and 11.

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.

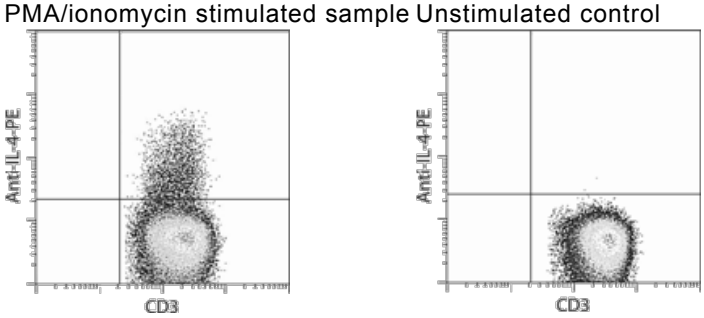
Examples of immunofluorescent staining

Human peripheral blood mononuclear cells (PBMCs), either unstimulated (left images) or stimulated with SEB for six hours. After two hours, brefeldin A was added for stimulated and unstimulated cells. The cells were fixed, permeabilized, and intracellularly stained with Anti-IL-4 antibodies as well as with CD4 (Vit4) antibodies and CD154 antibodies. Cells were then analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. CD4+ lymphocets were pregated for the analysis. Cell debris were excluded from the analysis based on scatter signals.



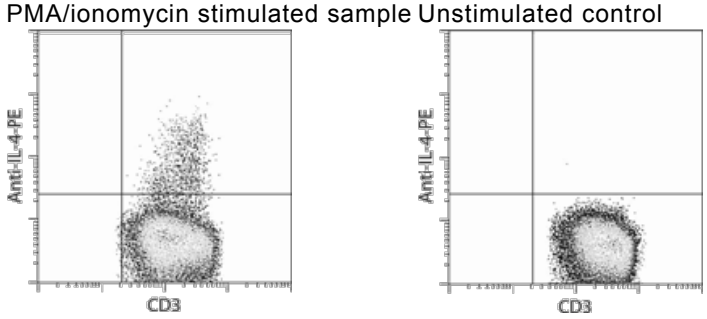
Rhesus monkey PBMCs were incubated with or without PMA/ionomycin and intracellularly stained with Anti-IL-4-PE. (Data were kindly provided by Dr. Paula Acierno, Harvard Medical School, Boston, USA.)

PMA/ionomycin stimulated sample Unstimulated control



Cynomolgus monkey PBMCs were incubated with or without PMA/ionomycin and intracellularly stained with Anti-IL-4-PE. (Data were kindly provided by Dr. Paula Acierno, Harvard Medical School, Boston, USA.)

PMA/ionomycin stimulated sample Unstimulated control



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