Anti-IL-17A 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-17A-FITC	for 30 tests	130-099-097
Anti-IL-17A-FITC	for 100 tests	130-094-520
Anti-IL-17A-PE	for 30 tests	130-099-420
Anti-IL-17A-PE	for 100 tests	130-094-521
Anti-IL-17A-APC	for 30 tests	130-099-202
Anti-IL-17A-APC	for 100 tests	130-094-519
Anti-IL-17A-VioBlue	for 30 tests	130-108-193
Anti-IL-17A-VioBlue	for 100 tests	130-097-018
Anti-IL-17A-PE-Vio615	for 30 tests	130-107-200
Anti-IL-17A-PE-Vio615	for 100 tests	130-107-145
Anti-IL-17A-PE-Vio770	for 30 tests	130-100-327
Anti-IL-17A-PE-Vio770	for 100 tests	130-096-748
Anti-IL-17A-APC-Vio770	for 30 tests	130-100-077
Anti-IL-17A-APC-Vio770	for 100 tests	130-096-656

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-17A
Clone	CZ8-23G1
Isotype	mouse IgG1
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	CTLA-8, IL-17
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.

Clone CZ8-23G1 detects human interleukin 17 (IL-17). Interleukin 17A (CTLA8), a member of the IL-17 family (IL-17A–F), is a disulfide-linked homodimeric glycoprotein. Human IL-17A consists of 155 amino acids with a molecular weight of around 35 kDa. IL-17A is produced by $CD4^+$ T helper cells, a third T cell subset termed TH17, which secrete also cytokines such as IL-17F and IL-22 and express the NK cell marker CD161. IL-17A secretion has also been described for other cell types, such as $CD8^+$ memory T cells. Furthermore, intracellular IL-17A has also been detected in eosinophils, neutrophils, and blood monocytes. Emerging data about TH17 cells suggest that these cells are involved in the recruitment of neutrophils to control early stages of infections to a number of pathogens, such as extracellular bacteria and fungi. IL-17A and TH17 cells have been shown to play an important role in immune-mediated diseases, such as rheumatoid arthritis, psoriasis, multiple sclerosis, asthma, inflammatory bowel diseases, and other immune-mediated inflammatory conditions.

Depending on the cytokine milieu present at time of the initial engagement, $CD4^+$ naive T cells can differentiate into various subsets (TH1, TH2, and TH17). For the differentiation into TH17 cells several cytokines have been described, including TGF-β, IL-1β, IL-6, IL-21, and IL-23. RORγt was identified as the master regulator gene for TH17 cells.

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

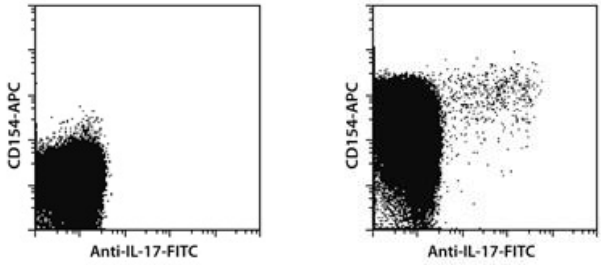
mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

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) were incubated with or without CytoStim for 6 hours. After 2 hours brefeldin A was added. The cells were harvested, fixed, permeabilized, and intracellularly stained with Anti-IL-17A antibodies. Cell surface staining was performed with CD4 and CD154 antibodies. Cells were analyzed using the MACSQuant® Analyzer. Gating was performed according to CD4 expression and side scatter properties of the cells. Cell debris was excluded from the analysis with propidium iodide.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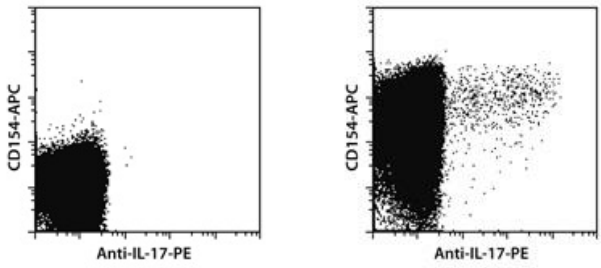
Anti-IL-17-FITC
CD154-APC
Anti-IL-17-FITC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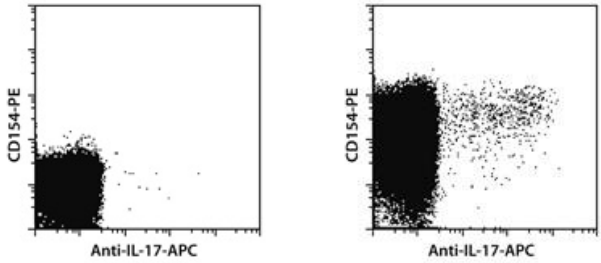
Anti-IL-17-PE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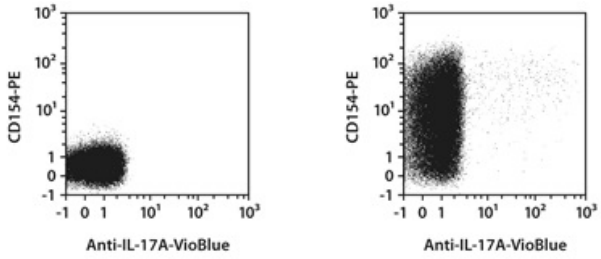
Anti-IL-17-APC
CD154-PE
Anti-IL-17-APC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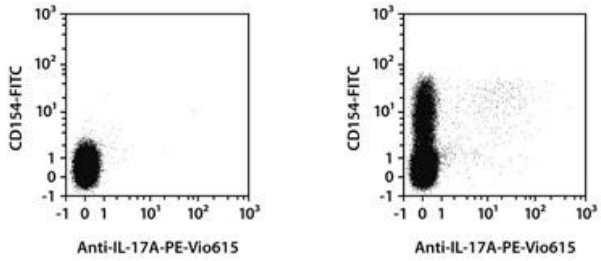
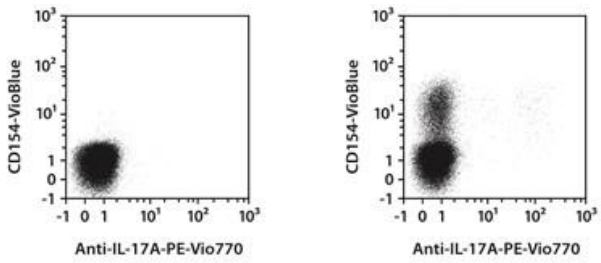
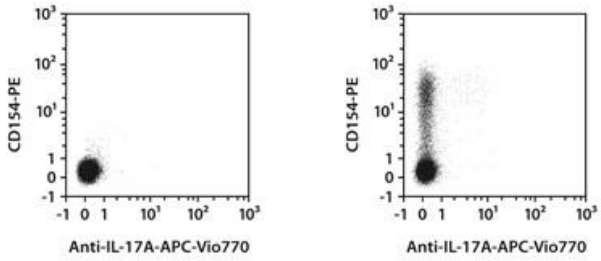
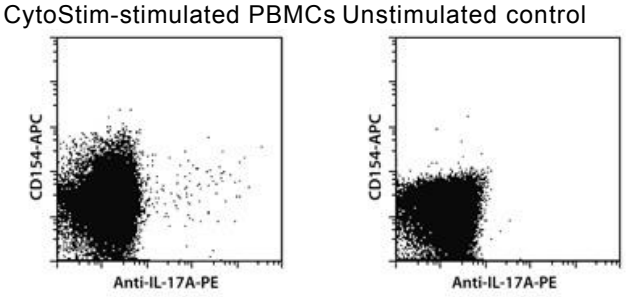
Anti-IL-17A-VioBlue
CD154-PE
Anti-IL-17A-VioBlue


CD154-FITC
Anti-IL-17A-PE-Vio615
CD154-FITC
Anti-IL-17A-PE-Vio615

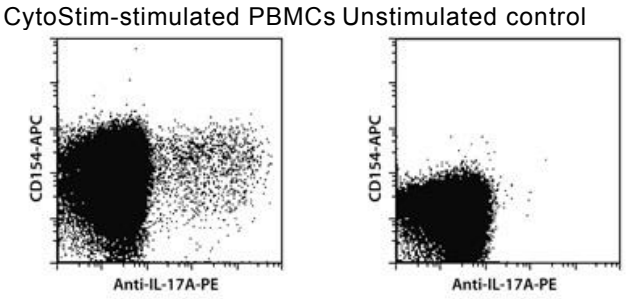

CD154-VioBlue
Anti-IL-17A-PE-Vio770
CD154-VioBlue
Anti-IL-17A-PE-Vio770



PBMCs from cynomolgus monkey were incubated with CytoStim for 6 hours or left untreated. After two hours, brefeldin A was added. The cells were fixed, permeabilized, and intracellularly stained with Anti-IL-17A-PE and CD154-APC. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer.



PBMCs from rhesus monkey were incubated with CytoStim for 6 hours or left untreated. After two hours, brefeldin A was added. The cells were fixed, permeabilized, and intracellularly stained with Anti-IL-17A-PE and CD154-APC. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer.



References

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8. **Ivanov, I. I.** (2006) The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17$^+$ T helper cells. Cell 126: 1121–1133.

Warranty

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