Miltenyi Biotec

Anti-IL-17A antibodies
human

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1. Description

Components	**1 mL Anti-IL-17A antibodies, human** conjugated to various dyes.	
	FITC	130-094-520
	PE	130-094-521
	APC	130-094-519
	PE-Vio770™	130-096-748
	APC-Vio770	130-096-656
Clone	CZ8-23G1 (isotype: mouse IgG1).	
Capacity	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.	

Cross-reactivity: The Anti-TIL-17A antibody has been tested to react with rhesus monkey (*Macaca mulatta*) cells.

1.1 Background information

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.[1] 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.[2] IL-17A secretion has also been described for other cell types, such as CD8+ memory T cells.[3] 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.[4]
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.[5,6,7] RORγt was identified as the master regulator gene for TH17 cells.[8]

1.2 Applications

- Identification and enumeration of IL-17A–producing cells by flow cytometry or fluorescence microscopy.
- Identification and enumeration of IL-17A–producing antigen-specific T cells upon restimulation with the respective antigen.
- Analysis of cytokine production of rare cells by solid-phase staining technology, in combination with magnetic enrichment of cells (refer to protocol 2.3.2).

1.3 Recommended antibody dilution

- Anti-IL-17A antibodies should be used at a dilution of 1:10.

1.4 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). 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.
- Culture medium, e.g., RPMI 1640 (# 130-091-440) containing 5% human serum, for example, autologous AB serum (do not use BSA or FBS because of non-specific stimulation!).
- Reagents for T cell stimulation, such as CytoStim (# 130-092-172, # 130-092-173) and CD40 pure – functional grade (# 130-094-133) antibody to avoid downregulation of CD154.
- Secretion inhibitor, e.g., brefeldin A.
- Inside Stain Kit (# 130-090-477) for fixation and permeabilization of cells.

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- (Optional) Fluorochrome-conjugated antibodies for cell surface staining, e.g., CD4-FITC (# 130-080-501), CD4-PE (# 130-091-231), or CD4-APC (# 130-091-232).
- Fluorochrome-conjugated antibodies for intracellular staining of activation markers, e.g., CD154-PE (# 130-092-289) or CD154-APC (# 130-092-290).

Additional requirements for intracellular cytokine staining in combination with magnetic cell separation (see protocol 2.3.2)

- MACS MicroBeads of choice, e.g., CD4 MicroBeads (# 130-045-101).
- MS Columns and suitable MACS Separator (MiniMACS™, OctoMACS™, VarioMACS™, or SuperMACS™ II Separator).

  ▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS or SuperMACS II Separators. For details refer to the respective MACS Separator data sheet.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

2. Protocols

2.1 Sample preparation

To detect and isolate cytokine-secreting cells, best results are achieved by starting the assay with fresh PBMCs or with other leukocyte-containing single-cell preparations from tissues or cell lines. Alternatively, frozen cell preparations can be used. For details refer to the protocols section at www.miltenyibiotec.com/protocols.

2.2 *In vitro* stimulation of T cells

▲ Always include a negative control in the experiment. The sample should be treated exactly the same as the stimulated sample, except for the addition of the stimulus.

▲ A positive control should also be included in the experiment.

▲ Do not use media containing any non-human proteins.

1. Wash cells by adding medium and centrifuge at 300×g for 10 minutes. Aspirate supernatant.
2. Resuspend cells at a density of 10^7 per mL in culture medium, containing 5% human serum. Plate cells in dishes at a density of 5×10^6 cells/cm^2. For details refer to section 5. Appendix: Flask and dish sizes for stimulation.
3. Add an antigen or control reagent in the appropriate concentration, for example, CytoStim (20 µL/mL).
4. Incubate cells for 2 hours at 37 °C and 5% CO_2.
5. Add 1 µg/mL brefeldin A and incubate for an additional 4 hours at 37 °C and 5% CO_2.
6. Collect cells carefully by pipetting up and down when working with smaller volumes or by using a cell scraper. Rinse the dish with cold buffer. Check microscopically for any remaining cells, if necessary, rinse the dish again.

2.3 Intracellular immunofluorescent cytokine staining protocols

2.3.1 Intracellular staining in suspension

▲ It is recommended to stain 10^6 cells per sample. When working with up to 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 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Wash up to 10^7 cells by adding 1–2 mL of buffer. 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. 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. Mix well and incubate for 20 minutes at room temperature.
5. Centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
6. Wash cells by adding 1 mL of buffer. Centrifuge at 300×g for 5 minutes and aspirate supernatant carefully.

   ▲ **Note:** Fixed cells may be stored in azide-containing buffer at 2–8 °C for up to three days.
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 and centrifuge for 5 minutes at 300×g. Aspirate supernatant carefully.
9. Resuspend cells in 90 µL of Inside Perm. Add 10 µL of Anti-IL-17A antibodies.
10. (Optional) Add additional staining antibodies to the solution, e.g., 10 µL of CD4-FITC (# 130-080-501) and 10 µL of CD154-APC (# 130-092-290).
11. Mix well and incubate for 10 minutes at room temperature.
12. Wash cells by adding 1 mL of Inside Perm. Centrifuge at 300×g for 5 minutes and aspirate supernatant carefully.
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.

2.3.2 Intracellular staining in combination with magnetic cell separation (solid phase intracellular staining)

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total 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 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through a 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ 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.

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▲ Always wait until the column reservoir is empty before proceeding to the next step.

1. Wash up to 10^7 nucleated cells by adding 1–2 mL of buffer. Centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
3. Add 20 µL of MACS MicroBeads of choice, e.g. CD4 MicroBeads (# 130-045-101) per 10^7 total cells.

   ▲ **Note:** For details on the procedure refer to the respective MACS MicroBead data sheet.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. (Optional) Stain cell surface antigens with antibodies that are sensitive to fixation according to the manufacturer's recommendations.
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 cells in 500 µL of buffer.
8. Place MS Column in the magnetic field of a suitable MACS Separator.
9. Prepare column by rinsing with 500 µL of buffer.
10. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
11. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with effluent from step 10.
12. Remove column from the separator and place it on a suitable collection tube.
13. Pipette 500 µL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
14. Add 500 µL of Inside Fix to the eluted cell fraction and incubate for 20 minutes at room temperature.
15. Place a second MS Column in the magnetic field of a suitable MACS Separator and prepare column by rinsing with 500 µL of buffer.
16. Apply the fixed cell suspension onto the column.
17. Wash cells by rinsing the column with 1×500 µL of buffer, followed by 2×500 µL of Inside Perm.
18. Prepare a solution of 10 µL of Anti-IL-17A antibodies and 90 µL of Inside Perm.
19. (Optional) Add additional staining antibodies to the solution, e.g., 10 µL of CD4-FITC (# 130-080-501) and 10 µL of CD154-APC (# 130-092-290).

    ▲ **Note:** Do not exceed a total volume of 150 µL.
20. Apply the solution onto the column and incubate for 10 minutes at room temperature.

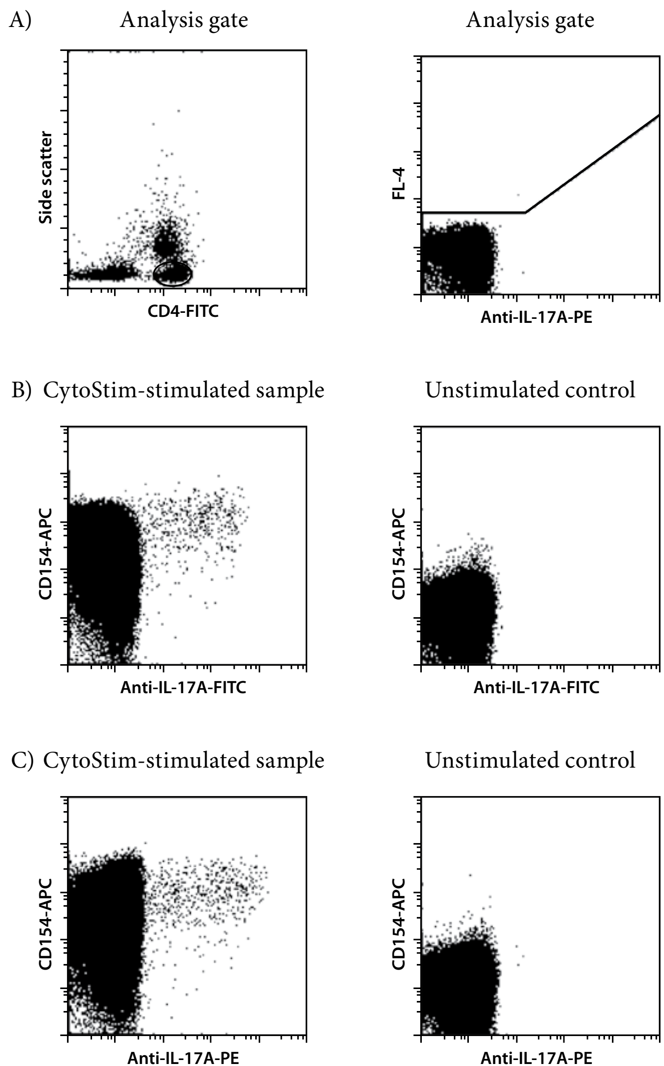
    ▲ **Note:** The MACS Column has a flow-stop mechanism that will retain the solution in the column.
21. Wash cells by rinsing the column with 2×500 µL of Inside Perm followed by 1×500 µL of buffer.
22. Remove column from the separator and place it on a suitable collection tube.
23. Pipette 500 µL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
24. Cells are now ready for analysis. 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.

3. Examples of immunofluorescent staining with Anti-IL-17A antibodies

Human 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 conjugated to FITC (B), PE (C), APC (D), PE-Vio770 (E), or APC-Vio770 (F). Cell surface staining was performed with CD4-FITC (# 130-080-501) or CD4-PE (# 130-091-231) and CD154-PE (# 130-092-289) or CD154-APC (# 130-092-290). 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 in a fluorescence 4 versus fluorescence 3 dot plot (A).



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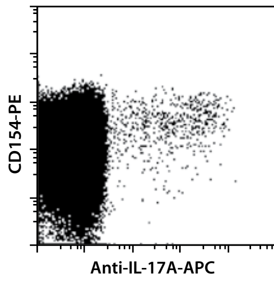
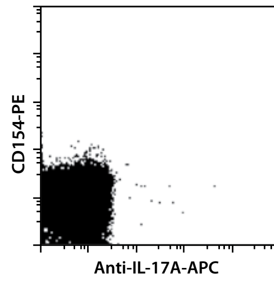
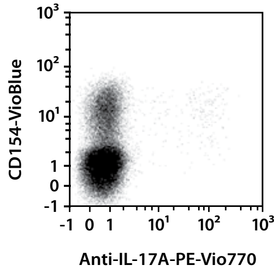
D) CytoStim-stimulated sample



Unstimulated control



E) CytoStim-stimulated sample



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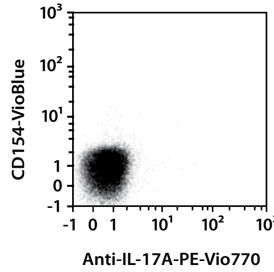
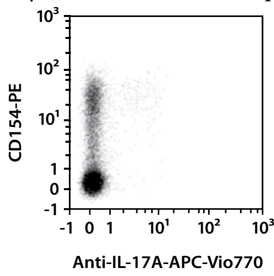
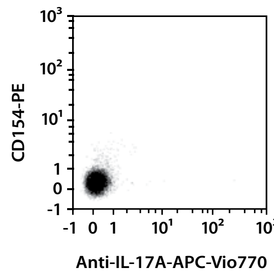


F) CytoStim-stimulated sample



Unstimulated control



5. References

1. Yao, Z. *et al.* (1995) Human IL-17: a novel cytokine derived from T cells. J. Immunol. 155: 5483–5486.
2. Cosmi, L. *et al.* (2008) Human interleukin 17-producing cells originate from a CD161⁺CD4⁺ T cell precursor. J. Exp. Med. 205: 1903–1916.
3. Kolls, J. K. and Linden, A. (2004) Interleukin-17 family members and inflammation. Immunity 21: 467–476.
4. Tesmer, L. A. *et al.* (2008) Th17 cells in human disease. Immunol. Rev. 223: 87–113.
5. Yang, L. *et al.* (2008) IL-21 and TGF-β are required for differentiation of human TH17 cells. Nature 454: 350–352.
6. Manel, N. *et al.* (2008) The differentiation of human TH-17 cells requires transforming growth factor-β and induction of the nuclear receptor RORγt. Nature Immunol. 9: 641–649.
7. Volpe, E. *et al.* (2008) A critical function for transforming growth factor-β, interleukin 23 and proinflammatory cytokines in driving and modulating human TH-17 responses. Nature Immunol. 9: 650–657.
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.

5. Appendix: Flask and dish sizes for *in vitro* stimulation of T cells

For *in vitro* stimulation of T cells (refer to 2.2) the cells should be resuspended in culture medium, containing 5% of human serum, at a dilution of 10^7 cells/mL. The cells should be plated at a density of 5×10^6 cells/cm². Both the dilution and the cell density are important to assure optimum stimulation.

The following table lists culture plate, dish and flask sizes suitable for different cell numbers. It also indicates the appropriate amount of medium to add.

Total cell number	Medium volume to add	Culture plate	Well diameter
0.15×10^7	0.15 mL	96 well	0.64 cm
0.50×10^7	0.50 mL	48 well	1.13 cm
1.00×10^7	1.00 mL	24 well	1.60 cm
2.00×10^7	2.00 mL	12 well	2.26 cm
5.00×10^7	5.00 mL	6 well	3.50 cm
Total cell number	**Medium volume to add**	**Culture dish**	**Dish diameter**
4.5×10^7	4.5 mL	small	3.5 cm
10.0×10^7	10.0 mL	medium	6 cm
25.0×10^7	25.0 mL	large	10 cm
50.0×10^7	50.0 mL	extra large	15 cm
Total cell number	**Medium volume to add**	**Culture flask**	**Growth area**
12×10^7	12 mL	50 mL	25 cm²
40×10^7	40 mL	250 mL	75 cm²
80×10^7	80 mL	720 mL	162 cm²
120×10^7	120 mL	900 mL	225 cm²

All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

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