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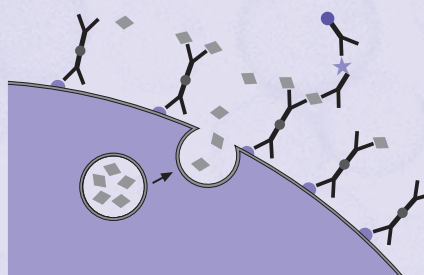
Miltenyi Biotec

IL-17 Secretion Assay – Cell Enrichment and Detection Kit (PE)

human

For 50 tests with 10^7 cells

Order no. 130-094-542



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Contents

1. Description

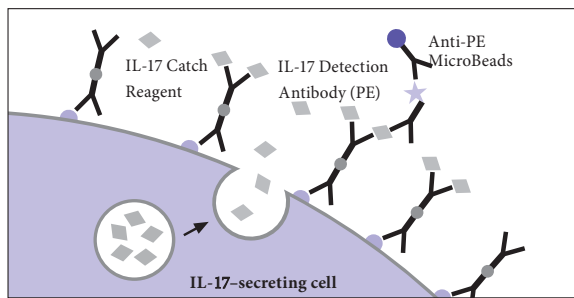
Contents

1. Description
 - 1.1 Principle of the IL-17 Secretion Assay
 - 1.2 Background information
 - 1.3 Applications
 - 1.4 Reagent and instrument requirements
2. Protocol overview
3. Experimental setup
 - 3.1 Controls
 - 3.2 Kinetics of restimulation and proposed time schedule
 - 3.3 Counterstaining of cytokine-secreting cells
 - 3.4 Two-color cytokine analysis
 - 3.5 Combination with peptide-MHC tetramer staining
 - 3.6 Detection without prior enrichment
4. Protocol for the IL-17 Secretion Assay
 - 4.1 Cell preparation
 - 4.2 *In vitro* stimulation
 - 4.3 Cytokine Secretion Assay
 - 4.4 Magnetic labeling
 - 4.5 Magnetic separation

5. Detection and analysis of IL-17-secreting T cells
6. References
7. Appendix: Flask and dish sizes for *in vitro* stimulation

1. Description

Components	1 mL IL-17 Catch Reagent: anti-IL-17A monoclonal antibody (mouse IgG1) conjugated to CD45-specific monoclonal antibody (mouse IgG2a). 1 mL IL-17 Detection Antibody (PE): anti-IL-17A monoclonal antibody (mouse IgG1) conjugated to R-phycoerythrin. 1 mL Anti-PE MicroBeads: colloidal super-paramagnetic MicroBeads conjugated to monoclonal mouse anti-PE antibody (mouse IgG1).
Size	For 50 tests with 10^7 cells.
Product format	All components 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 dates are indicated on the vial labels.



1.1 Principle of the IL-17 Secretion Assay

Antigen-specific T cells are analyzed using the IL-17 Secretion Assay starting from whole blood, peripheral blood mononuclear cells (PBMCs), or other leukocyte containing single-cell preparations. The cells are restimulated for a short period of time with a polyclonal stimulus such as CytoStim or specific peptide, protein, or other protein antigen preparations, e.g., from *Candida albicans* (*C. albicans*).

Subsequently, an IL-17-specific **Catch Reagent** is attached to the cell surface of all leukocytes. The cells are then incubated for a short time at 37 °C to allow cytokine secretion. The secreted IL-17 binds to the IL-17 Catch Reagent on the positive, secreting cells. These cells are subsequently labeled with a second IL-17-specific antibody, the **IL-17 Detection Antibody** conjugated to PE for sensitive detection by flow cytometry.

The IL-17-secreting cells can now be magnetically labeled with **Anti-PE MicroBeads** and enriched over a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled cells are retained within the column. The unlabeled cells run through. After removing the column from the magnetic field, the magnetically retained IL-17-secreting cells can be eluted as positively selected cell fraction. The cells can now be used for cell culture or analysis. Since viable cells are analyzed, non-specific background can be minimized by dead cell exclusion. This provides highest sensitivity of analysis.

1.2 Background information

Interleukin 17 (IL-17, IL-17A, CTLA8), a member of the IL-17 family (IL-17A-F), is a disulfide-linked homodimeric glycoprotein. Human IL-17 consists of 155 amino acids with a molecular weight of around 35 kDa.¹ IL-17 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-17 secretion has also been described for other cell types, such as CD8⁺ memory T cells.³ Furthermore, intracellular IL-17 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-17 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-1 β , IL-6, IL-21, and IL-23.^{5,6,7} ROR γ t was identified as a master regulator gene for Th17 cells.⁸

1.3 Applications

- Detection and enrichment of viable IL-17-secreting leukocytes.
- Detection and enrichment of viable IL-17-secreting leukocytes from whole blood. The IL-17 Secretion Assay can be started directly from whole blood. For details on the procedure refer to www.miltenyibiotec.com/protocols.
- Detection and enrichment of IL-17-secreting, antigen-specific T cells for enumeration and phenotypic characterization as well as for expansion and functional characterization.
- Monitoring and analysis of antigen-specific T cell immunity, e.g., bacterial and fungal immunity, autoimmunity, and other inflammatory disorders.
- Isolation and expansion of antigen-specific T cells.
- Enrichment and analysis of IL-17-secreting cells for determination of functional antigens in disease and for T cell receptor (TCR) epitope mapping.
- Analysis or cloning of TCR repertoire of antigen-specific T cells.

1.4 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). Degas buffer before use, as air bubbles could block the column.
 - ▲ **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 Ca²⁺ or Mg²⁺ are not recommended for use.
- **Culture medium, e.g., RPMI 1640 (# 130-091-440),** containing 5% human serum, like autologous or AB serum (do not use BSA or FBS because of non-specific stimulation!).
- **(Optional) Cell stimulation reagents, e.g., CytoStim (# 130-092-172, # 130-092-173).** For details see the respective data sheet. For more information about other stimulation reagents see www.miltenyibiotec.com.
- **(Optional) For detection of activated T cells with CD154,** the incubation with CD40 pure – functional grade (# 130-094-133) is recommended to avoid downregulation of CD154 expression.
- **Propidium Iodide Solution (# 130-093-233) or 7-AAD** for flow cytometric exclusion of dead cells without fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

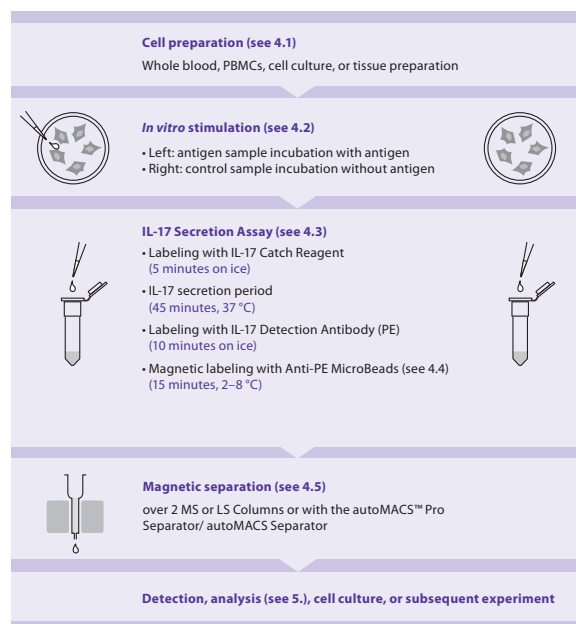
- **(Optional) Staining reagents** such as CD154-APC (# 130-092-290), CD4-FITC (# 130-080-501), CD161-PE (#130-092-677), or CD161-APC (# 130-092-678).
- **MACS Columns and MACS Separators:**

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10 ⁷	2×10 ⁸	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10 ⁸	2×10 ⁹	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
autoMACS	2×10 ⁸	4×10 ⁹	autoMACS Pro, autoMACS

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- **Refrigerated centrifuge (2–8 °C).**
- **Rotation device for tubes: MACSmix™ Tube Rotator (# 130-090-753).**
- **(Optional) Pre-Separation Filters (# 130-041-407)** to remove cell clumps.

2. Protocol overview



3. Experimental setup

3.1 Controls

Negative control

For accurate detection of IL-17-secreting cells, a negative control sample should always be included. This will provide information about IL-17 secretion unrelated to the specific antigen-stimulation, but, e.g., due to ongoing *in vivo* immune responses. The control sample should be treated exactly the same as the antigen-stimulated sample except for the addition of antigen, or by using a control antigen.

Positive control

When setting up a new experiment, it is recommended to include a positive control. As a positive control, a sample stimulated with CytoStim (# 130-092-172, # 130-092-173) 20 µL/mL for 4–6 hours, may be included in the experiment.

3.2 Kinetics of restimulation and proposed time schedule

Peptides

Upon stimulation with peptide, the cells can be analyzed for IL-17 secretion 3–6 hours later.

Proteins

Upon stimulation with protein antigen preparations, e.g., from *C. albicans*, the cells can be analyzed for IL-17 secretion 8–16 hours later.

It is possible to start the stimulation of the cells late in the afternoon and perform the IL-17 Secretion Assay the following morning.

3.3 Counterstaining of cytokine-secreting cells

The IL-17-secreting cells are stained with PE-conjugated IL-17 Detection Antibodies. To identify cells of interest, counterstaining for T cells with, e.g., CD4-FITC (# 130-080-501) and CD154-APC (# 130-092-290) is important.

▲ Do not use tandem conjugates of phycoerythrin, like Cy[™]-Chrome, PE-Cy5, ECD, or PC5. They may also be recognized by the Anti-PE MicroBeads.

▲ Upon activation of T cells, TCR, and some associated molecules, like CD3, might be down-regulated.

▲ The samples should be stained with propidium iodide (PI) or 7-AAD prior to acquisition, to exclude dead cells from analysis. This will reduce non-specific background staining and increase sensitivity.

▲ For optimal sensitivity, we recommend labeling of undesired non-T cells such as monocytes with antibodies conjugated to Peridinin-chlorophyll-protein (PerCP), e.g., CD14-PerCP. These cells together with PI stained dead cells can then be excluded by gating.

3.4 Two-color cytokine analysis

IL-17-secreting cells can be analyzed simultaneously for IFN- γ , IL-2, IL-10, or TNF- α production by two-color cytokine analysis combining the IL-17 Secretion Assay with the IFN- γ Secretion Assay – Detection Kit (APC) (# 130-090-762) or IFN- γ Secretion Assay – Detection Kit (FITC) (# 130-090-433), IL-2 Secretion Assay – Detection Kit (APC) (# 130-090-763), IL-10 Secretion Assay – Detection Kit (APC) (# 130-090-761), or TNF- α Secretion Assay – Detection Kit (APC) (# 130-091-267). Detailed protocols are included in the data sheets of the Cytokine Secretion Assay – Detection Kits (APC) and are also available at www.miltenyibiotec.com/protocols.

3.5 Combination with peptide-MHC tetramer staining

IL-17-secreting cells can be analyzed simultaneously for peptide-MHC tetramers combining the IL-17 Secretion Assay – Detection Kit (PE) with APC-conjugated peptide-MHC tetramers. A special protocol is available at www.miltenyibiotec.com/protocols.

3.6 Detection without prior enrichment

(Optional) If the sample contains more than 0.01–0.1% of IL-17-secreting cells, you may be able to analyze IL-17-secreting cells without prior enrichment (see also: IL-17 Secretion Assay – Detection Kit (PE), # 130-094-537, or IL-17 Secretion Assay – Detection Kit (APC), # 130-094-536). The assay can also be performed directly starting from whole blood. For details on the procedure refer to www.miltenyibiotec.com/protocols.

4. Protocol for the IL-17 Secretion Assay

4.1 Cell 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.

▲ **Note:** PBMCs may be stored overnight. The cells should be resuspended and incubated in culture medium as described in 4.2 step 2.

▲ **Note:** Remove platelets after density gradient separation. Resuspend cell pellet, fill tube with buffer, and mix. Centrifuge at 200 \times g for 10–15 minutes at 20 °C. Carefully remove supernatant.

Special protocols for whole blood: You can start the IL-17 Secretion Assay directly from whole blood. For details on the procedure refer to www.miltenyibiotec.com/protocols.

4.2 *In vitro* stimulation

▲ Always include a negative control in the experiment. A positive control may also be included (see 3.1).

▲ Do not use media containing any non-human proteins, such as BSA or FBS, as they lead to non-specific stimulation.



Protocol for *in vitro* stimulation

1. Wash cells by adding medium, centrifuge at 300 \times g for 10 minutes.
2. Resuspend cells in culture medium, containing 5% human serum, adjust to 10⁷ cells/mL and 5 \times 10⁶ cells/cm² (see 7. Appendix: Flask and dish sizes for stimulation).
3. Add antigen or control reagent:

CytoStim: 4–6 hours at 37 °C, 5–7% CO₂, e.g. 20 μ L/mL
 Peptide: 3–6 hours at 37 °C, 5–7% CO₂, e.g., 1–10 μ g/mL
 Protein preparation: 8–16 hours at 37 °C, 5–7% CO₂, e.g., 20 μ g/mL

For comparison of different experiments, the stimulation time should always be the same (see 3.2).

▲ **Note:** If CD154 antibodies are used in the labeling step of the cytokine secretion assay to stain activated CD4⁺ T cells, a CD40-blocking antibody has to be added during the *in vitro* stimulation step to prevent CD154 down-regulation.

4. Collect cells carefully by using a cell scraper or by pipetting up and down when working with smaller volumes. Rinse the dish with cold buffer. Check microscopically for any remaining cells, if necessary, rinse the dish again.

4.3 Cytokine Secretion Assay

General considerations

▲ The assay is optimized for cell samples containing <1% of total IL-17-secreting cells. If ≥1% of IL-17-secreting cells are expected, it is necessary to dilute the cells further during the cytokine secretion period, and therefore a larger test tube will be needed (see table below). The dilution prevents non-specific staining of cells not secreting IL-17 during this period.

▲ For each test with 10^7 total cells, prepare:

- 50 mL of **cold buffer** (2–8 °C)
- 100 μL of **cold medium** (2–8 °C)
- 10 mL (or 100 mL; see table below) of **warm medium** (37 °C).

▲ 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 (exception: warm medium during secretion period).

▲ Volumes 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).

▲ Do not remove supernatant by decanting. This will lead to cell loss and incorrect incubation volumes. Aspirate supernatant.

▲ Dead cells may bind non-specifically to MACS MicroBeads or antibodies. Therefore, when working with cell preparations containing large amounts of dead cells, they should be removed before starting the IL-17 Secretion Assay, e.g., by density gradient centrifugation or by using the Dead Cell Removal Kit (# 130-090-101).



Labeling cells with IL-17 Catch Reagent

1. Use 10^7 total cells in a 15 mL closable tube per sample.
2. Wash cells by adding 10 mL of **cold buffer**, centrifuge at $300 \times g$ for 5 minutes at 2–8 °C, aspirate supernatant completely.
3. Resuspend cell pellet in 80 μL of **cold medium** per 10^7 total cells.
4. Add 20 μL of **IL-17 Catch Reagent** per 10^7 total cells, mix well and incubate for 5 minutes on ice.



IL-17 secretion period

1. Add **warm** (37 °C) medium to dilute the cells according to the following table:

Expected number of IL-17-secreting cells	Dilution	Amount of medium to add per 10^7 total cells
<1%	10^6 cells/mL	10 mL
≥1%	$\leq 10^5$ cells/mL	100 mL

▲ **Note:** For frequencies of cytokine-secreting cells >20% the cells need to be further diluted, e.g., by a factor of 5.

2. Incubate cells in closed tube for 45 minutes at 37 °C under slow continuous rotation using the MACSmix Tube Rotator (# 130-090-753), or turn tube every 5 minutes to resuspend settled cells.

▲ **Note:** During this step it is crucial to prevent contact of cells to avoid cross contamination with cytokines.



Labeling cells with IL-17 Detection Antibody (PE)

1. Put the tube on ice.
2. Wash the cells by filling up the tube with **cold buffer** and centrifuge at $300 \times g$ for 5 minutes at 2–8 °C. Aspirate supernatant completely.
 - ▲ **Note:** If the volume of the cell suspension was higher than the volume of the added buffer, then repeat the wash step.
3. Resuspend cell pellet in 80 μL of **cold buffer** per 10^7 total cells.
4. Add 20 μL of **IL-17 Detection Antibody (PE)** per 10^7 total cells.
5. (Optional) Add staining antibodies, e.g., 10 μL of CD4-FITC and 10 μL of CD154-APC.
6. Mix well and incubate for 10 minutes on ice.
7. Add 10 mL of **cold buffer** and centrifuge at $300 \times g$ for 10 minutes at 2–8 °C. Aspirate supernatant completely.
8. Proceed to magnetic labeling (4.4).

4.4 Magnetic labeling

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

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



Magnetic labeling with Anti-PE MicroBeads

1. Resuspend cell pellet in 80 µL of **cold buffer** per 10^7 total cells.
2. Add 20 µL of **Anti-PE MicroBeads** per 10^7 total cells.
3. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
4. Wash cells by adding 10 mL of **cold buffer** per 10^7 total cells and centrifuge at 300×g for 10 minutes at 2–8 °C. Aspirate supernatant completely.
5. Resuspend cell pellet in 500 µL of **cold buffer**. For higher cell numbers than 5×10^7 use a dilution of 10^8 cells/mL.
6. (Optional) Take an aliquot for flow cytometric analysis and cell count of the fraction before enrichment.
6. Proceed to magnetic separation (4.5).

4.5 Magnetic separation



Magnetic separation using MS or LS Columns

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of IL-17–secreting cells. For details see table in section 1.4.

▲ When enriching antigen-specific T cells, **always perform two consecutive column runs** to achieve best results.

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the appropriate amount of buffer:
MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.

MS: 3×500 µL LS: 3×3 mL

5. Remove column from the separator and place it on a suitable collection tube.

▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.

6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

MS: 1 mL LS: 5 mL

7. To increase the purity of IL-17–secreting cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

▲ **Note:** For subsequent cell culture, the cells can also be eluted with medium. If part of the cells are analyzed by flow cytometry, the medium should not contain phenol red.

11. Proceed to analysis (see section 5.), cell culture, or other subsequent experiment.



Magnetic separation with the autoMACS™ Pro Separator or the autoMACS™ Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Pro Separator or the autoMACS Separator.

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS Separator should have a temperature of ≥ 10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS™ Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:
Positive selection: “Posseld”
Collect positive fraction in row C of the tube rack.
4. Proceed to analysis (5.), cell culture, or other subsequent experiment.

Magnetic separation with the autoMACS™ Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos2.

- For a standard separation choose the following program:
Positive selection: "Posseld"
Collect positive fraction from outlet port pos2.
- Proceed to analysis (5.), cell culture, or other subsequent experiment.

5. Detection and analysis of IL-17-secreting T cells

▲ Add propidium iodide (PI) or 7-AAD to a final concentration of 0.5 µg/mL just prior to acquisition to exclude dead cells from flow cytometric analysis. Incubating with PI for longer periods will affect the viability of the cells.

Do not fix the cells when using PI or 7-AAD.

▲ For optimized sensitivity, an appropriate number of viable cells has to be acquired from the antigen stimulated sample as well as from the control sample.

▲ Note: Acquire 2×10^5 viable cells from each sample.

To illustrate the analysis, we describe the detection of IL-17-secreting T cells using the IL-17 Secretion Assay. The detailed description, including how to set gates, should serve as a model for the analysis of your own sample.

- 10^7 human PBMCs have been incubated for 4 hours with and without CytoStim (20 µL/mL) and CD40 pure – functional grade (1 µg/mL).

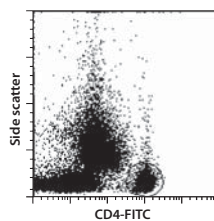
- The IL-17 Secretion Assay was performed on the stimulated and the unstimulated sample.
- Counterstaining of T cells was performed using CD4-FITC and CD154-APC.
- Dead cells were stained with PI, which was added just prior to flow cytometric analysis to a final concentration of 0.5 µg/mL.
- 200,000 viable cells of the fractions before enrichment and the complete enriched fractions were acquired by flow cytometry, from the stimulated as well as from the unstimulated samples.
- A lymphocyte gate based on CD4⁺ staining and side scatter properties was activated prior to further gating to exclude debris (A).
- Dead cells were excluded according to PI-staining (B).

▲ Note: The dead cell exclusion is crucial for the analysis of rare antigen-specific T cells, as dead cells may bind non-specifically to MicroBeads or antibodies. This could lead to false positive events.

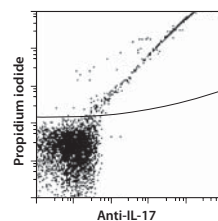
▲ Note: The sensitivity of detection is further enhanced by exclusion of undesired non-T cells, like monocytes which may cause non-specific background staining.

- Analysis of secreted IL-17 (PE) versus CD154-APC staining of CD4⁺ viable lymphocytes is displayed (C).

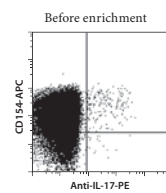
A) CD4⁺ lymphocyte gate



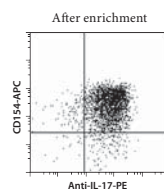
B) Dead cell exclusion



C) IL-17-secreting CD4⁺ T cells after stimulation with CytoStim



0.46% of the total CD4⁺ T cell population secrete IL-17 (see formula below).



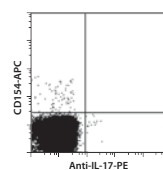
The IL-17-secreting CD4⁺ T cells have been enriched to 91.58%.

1354 IL-17⁺CD4⁺ T cells were enriched from 10^6 CD4⁺ cells (= 0.14%; see formula below).

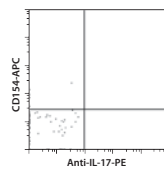
$$\% \text{ IL-17}^+ \text{ cells among CD4}^+ = \frac{\# \text{ of IL-17}^+ \text{ CD4}^+ \text{ cells in the analyzed sample}}{\# \text{ of total CD4}^+ \text{ cells in the analyzed sample}} \times 100$$

$$\% \text{ IL-17}^+ \text{ cells among CD4}^+ = \frac{\text{abs. \# of IL-17}^+ \text{ CD4}^+ \text{ cells in the enriched fraction}}{\text{abs. \# of total CD4}^+ \text{ cells before enrichment}} \times 100$$

Unstimulated control sample



0.02% of the total CD4⁺ T cell population secrete IL-17.



No IL-17⁺CD4⁺ T cell was enriched from 10^6 CD4⁺ cells (0.00%).

6. References

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7. Appendix: Flask and dish sizes for *in vitro* stimulation

For *in vitro* stimulation (see 4.2) 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 cm ²
40×10^7	40 mL	250 mL	75 cm ²
80×10^7	80 mL	720 mL	162 cm ²
120×10^7	120 mL	900 mL	225 cm ²

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.

Warranty

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