Anti-IL-6 antibodies
human

Contents

1. Description

Components

1 mL Anti-IL-6 antibodies, human
conjugated to various dyes.

PE	130-096-086
APC	130-096-088

or

0.5 mL Anti-IL-6 antibodies, human
pure – functional grade 130-096-093

Clone
MQ2-13A5 (isotype: rat IgG1).

Capacity
100 tests or up to 10^9 total cells.

The functional grade antibody is supplied at a concentration of 1 mg/mL.

Product format
Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Functional grade antibodies are supplied in phosphate-buffered saline (PBS), pH 7.2. Endotoxin levels have been tested and do not exceed 0.01 ng/µg of protein.

The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.

Storage
Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

Interleukin 6 (IL-6), originally identified as a B cell differentiation factor, is a multifunctional cytokine which regulates immune responses, hematopoiesis, acute phase responses, and inflammatory reactions. It induces the terminal maturation of activated B cells into antibody-secreting plasma cells and acts in synergy with IL-3 to support the proliferation of hematopoietic stem cells. IL-6 is produced by many cell types, such as monocytes, fibroblasts, endothelial cells, eosinophils, and T cells. Disturbed IL-6 production has been associated with pathological processes, including inflammatory autoimmune diseases and cancer.

1.2 Applications

- Flow cytometric identification and enumeration of IL-6–producing cells upon stimulation, e.g., in macrophages upon LPS treatment.
- The Anti-IL-6 antibody pure – functional grade is suited for functional assays, e.g. neutralization of IL-6 activity.

1.3 Recommended antibody dilution

- Anti-IL-6 antibodies conjugated to PE or APC 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!).
- Liposaccharide (LPS) for cell stimulation.
- Secretion inhibitor, e.g., brefeldin A.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells.
- (Optional) Fluorochrome-conjugated antibodies for cell surface staining, e.g., CD14-FITC (# 130-080-701). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

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2. Protocols

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

2.2 *In vitro* stimulation of PBMCs

▲ Always include a negative control in the experiment. The sample should be treated in exactly the same manner 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, such as BSA or FBS, because of non-specific stimulation.

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/cm². For details refer to section 4. Appendix: Flask and dish sizes for *in vitro* stimulation of PBMCs.
3. Add 100 µg/mL LPS.
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 5–7% 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

▲ 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 nucleated 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 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, e.g. add 10 µL of CD14-FITC and incubate for 10 minutes. 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 70 µL of Inside Perm. Add 20 µL of FcR Blocking Reagent. Mix well and incubate for 5 minutes.
10. Add 10 µL of the Anti-IL-6 antibody.
11. (Optional) Add additional staining antibodies to the solution, for example, for antigens which are internalized upon cell activation.

    ▲ **Note:** For efficient permeabilization upon intracellular staining the volume of Inside Perm should be at least 5× the volume of staining antibodies.
12. Mix well and incubate for 10 minutes in the dark at room temperature.
13. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. Aspirate supernatant carefully.
14. 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.

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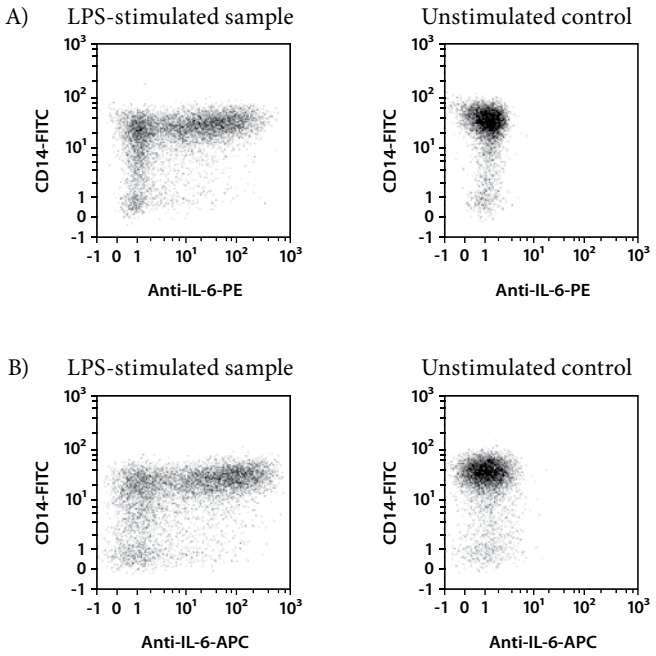
3. Examples of immunofluorescent staining with Anti-IL-6 antibodies

Human PBMCs were incubated with or without LPS for six hours. After two hours, brefeldin A was added. Cells were harvested, then stained with CD14-FITC (# 130-080-701), fixed, permeabilized, and intracellularly stained with Anti-IL-6 antibodies conjugated to PE (A) or APC (B). Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Gating was performed on monocytes according to scatter properties of the cells. Autofluorescent cell debris was excluded from the analysis in a fluorescence 4 versus fluorescence 3 dot plot.



4. Appendix: Flask and dish sizes for *in vitro* stimulation of PBMCs

For *in vitro* stimulation of PBMCs (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.

Warranty

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