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## 1. Description

<b>Components</b>	<p><b>1 mL Anti-IL-6 antibodies, mouse</b> conjugated to various dyes.</p> <table border="0"> <tr> <td>PE</td> <td>130-096-139</td> </tr> <tr> <td>APC</td> <td>130-096-131</td> </tr> </table> <p>or</p> <p><b>0.5 mL Anti-IL-6 antibodies, mouse</b> pure – functional grade      130-096-130</p>	PE	130-096-139	APC	130-096-131
PE	130-096-139				
APC	130-096-131				
<b>Clone</b>	MP5-20F3 (isotype: rat IgG1).				
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.  The functional grade antibody is supplied at a concentration of 1 mg/mL.				
<b>Product format</b>	<p>Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.</p> <p>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.</p> <p><i>The functional grade product contains no preservative and is sterile filtered; always handle under aseptic conditions.</i></p>				
<b>Storage</b>	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.
- Identification and enumeration of IL-6-producing antigen-specific T cells upon restimulation with the respective antigen or detection of IL-6-producing T cells upon polyclonal restimulation.
- Analysis of cytokine production of rare cells by solid-phase staining technology, in combination with magnetic enrichment of cells. For details see section 2.3.2.
- 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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- Culture medium, e.g., RPMI 1640 (# 130-091-440) containing 5% mouse serum (do not use BSA or FBS because of non-specific stimulation!).
- Liposaccharide (LPS) for cell stimulation.
- Secretion inhibitor, e.g., brefeldin A.
- Inside Stain Kit (# 130-090-477) for the fixation and permeabilization of cells.
- (Optional) Fluorochrome-conjugated antibodies for cell surface staining, e.g., CD115-PE (# 130-096-308) or CD115-APC (# 130-096-310). For more information about antibodies refer to [www.miltenyibiotec.com/antibodies](http://www.miltenyibiotec.com/antibodies).
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor-mediated antibody labeling.

## 2. Protocols

### 2.1 Sample preparation

Prepare peritoneal cavity cells by from a mouse injected with thioglycollate five days before. When working with other lymphoid organs, non-lymphoid tissues, or peripheral blood, prepare a single-cell suspension using manual methods or the gentleMACS™ Dissociator.

For details refer to the protocols section at [www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols).

### 2.2 *In vitro* stimulation of peritoneal macrophages

▲ 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-mouse 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<sup>6</sup> per mL in culture medium containing 5% mouse serum. Plate cells in dishes at a density of 5×10<sup>6</sup> cells/cm<sup>2</sup>.
3. (Optional) Remove non-adherent cells by exchanging the cell culture medium.
4. Add 100 ng/mL LPS.
5. Incubate cells for 2 hours at 37 °C and 5% CO<sub>2</sub>.
6. Add 1 µg/mL brefeldin A and incubate for an additional 4 hours at 37 °C and 5% CO<sub>2</sub>.
7. 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.

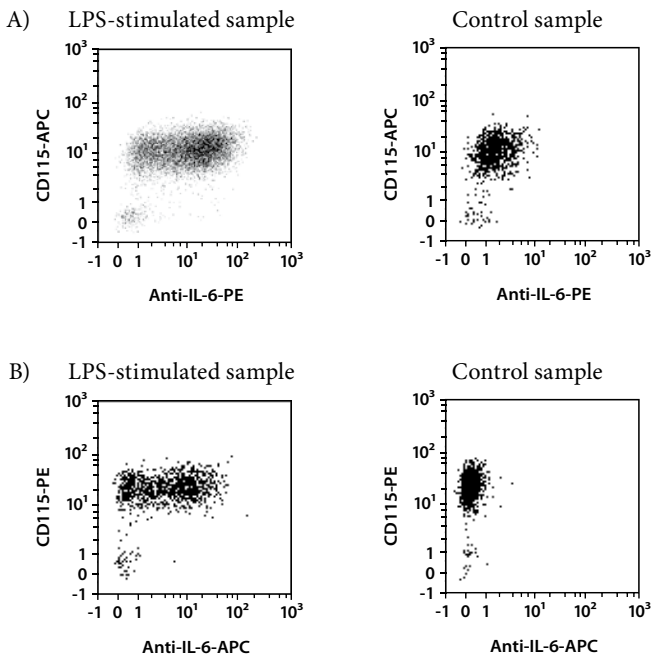
### 2.3 Intracellular staining of cells in suspension

▲ It is recommended to stain 10<sup>6</sup> cells per sample. When working with up to 10<sup>7</sup> 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<sup>7</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Wash up to 10<sup>7</sup> 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 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<sup>7</sup> 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 Anti-IL-6 antibody.
10. (Optional) Add additional staining antibodies to the solution, for example, 10 µL of CD115-PE (# 130-096-308).  
**▲ Note:** For efficient permeabilization upon intracellular staining the volume of Inside Perm should be at least 5× the volume of staining antibodies.
11. Mix well and incubate for 10 minutes in the dark at room temperature.
12. Wash cells by adding 1 mL of Inside Perm and centrifuge at 300×g for 5 minutes. 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.

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

Peritoneal cells were isolated from Balb/c mouse injected with thioglycollate five days before. Adherent cells were stimulated with LPS for six hours. After two hours brefeldin A was added. The cells were harvested, fixed, permeabilized, and intracellularly stained with Anti-IL-6 conjugated to PE (A) or APC (B) as well as with CD115-APC or CD115-PE. IL-6 staining was controlled by incubating cells with 100 µg/mL of Anti-IL-6 antibody, pure – functional grade prior to labeling with fluorochrome conjugated antibodies. Cells were analyzed by flow cytometry using the MACSQuant® Analyzer. Gating was performed according to forward scatter and side scatter properties of the cells. Cell debris were excluded from the analysis in a fluorescence 3 versus fluorescence 4 dot plot.



#### 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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