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## Product Information

### Anti-Interleukin-17

produced in goat, affinity isolated antibody

Catalog Number **I7767**

#### Product Description

Anti-Interleukin-17 (IL-17) was produced in goat using as immunogen recombinant human IL-17, expressed in *E. coli*. Affinity isolated antibody is obtained from goat anti-human IL-17 antiserum by immunospecific purification which removes essentially all goat serum proteins, including immunoglobulins, which do not specifically bind to human interleukin-17.

Anti-Interleukin-17 recognizes human IL-17 by various immunochemical techniques including neutralization, immunoblotting, ELISA, immunocytochemistry, and flow cytometry. By ELISA, this antibody shows ~10% cross-reactivity with recombinant mouse IL-17 and less than 1% cross-reactivity with recombinant human IL-17B.

Interleukin 17, also known as CTLA-8, is a T cell-derived hematopoietic cytokine. It was originally cloned from a T cell hybridoma produced by fusion of a mouse cytotoxic T cell clone and a rat T lymphoma.<sup>1</sup> IL-17 exhibits multiple biological activities on a variety of cells including: the induction of IL-6, IL-8 and G-CSF production in fibroblasts;<sup>2,3</sup> the enhancement of surface expression of ICAM-1 in fibroblasts;<sup>4</sup> activation of NF- $\kappa$ B<sup>5</sup> and costimulation of T cell proliferation.<sup>3</sup> IL-17 is an approximately 16 kDa polypeptide of 136 amino acids. The precursor form of IL-17 consists of 155 amino acids. To generate the mature IL-17 (136 amino acids), the precursor cleaves a 19 amino acid signal peptide. Human IL-17 shows approximately 62.5% amino acid homology to mouse IL-17 and 58% amino acid homology to rat IL-17.<sup>6</sup>

#### Reagent

Supplied as a lyophilized powder from a 0.2  $\mu$ m filtered solution in phosphate buffered saline, pH 7.4, containing 5% trehalose.

#### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

#### Storage/Stability

Prior to reconstitution, store at  $-20^{\circ}\text{C}$ . Reconstituted antibody may be stored at  $2-8^{\circ}\text{C}$  for up to one month. For prolonged storage, freeze in working aliquots. Avoid repeated freezing and thawing. Do not store in a frost-free freezer.

#### Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2  $\mu$ m filtered phosphate buffered saline to produce a 100  $\mu\text{g}/\text{mL}$  stock solution of the antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

#### Product Profile

Anti-Interleukin-17 has the ability to neutralize the biological activity of recombinant human IL-17 using the normal human dermal fibroblast (NHDF) cell line. In this bioassay, 25 ng/mL recombinant human IL-17 was mixed with various concentrations of the antibody for 1 hour at  $37^{\circ}\text{C}$  in a 96 well plate. After preincubation, cells (normal human dermal fibroblasts at  $0.5 \times 10^5/\text{mL}$ ) were added to the antigen-antibody mixture (total volume of 100  $\mu\text{L}$ ). The assay mixture was incubated at  $37^{\circ}\text{C}$  for 2 days in a humidified  $\text{CO}_2$  incubator. After incubation, 25  $\mu\text{L}$  of supernatant was collected from each well and assayed for human IL-17 levels using an IL-17 ELISA kit.

The  $\text{ND}_{50}$  of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of bioactivity of recombinant human IL-17 on a responsive cell line, when that cytokine is present at a concentration just high enough to elicit a maximum response.

Immunoblotting: a working concentration of 0.1-0.2 µg/ml is recommended. The detection limit for recombinant human IL-17 is ~5 ng/lane under non-reducing and reducing conditions.

ELISA: a working concentration of 0.5-1.0 µg/ml is recommended. The detection limit for recombinant human IL-17 is ~0.2 ng/well.

Immunocytochemistry: a working concentration of 2-15 µg/mL is recommended using cultured cells.

Flow cytometry: this antibody has been tested in PBMCs. For intracellular staining to detect IL-17, cells must be first fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in phosphate buffered saline. Dilute this antibody to 50 µg/mL and add 10 µL of the diluted solution to 1-5 x 10<sup>5</sup> cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled polyclonal antibodies can be visualized by a secondary reagent such as anti-goat IgG conjugated to a fluorochrome.

**Note:** In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration test.

#### References

1. Rouvier, E., et al., J. Immunol., **150**, 5445 (1993).
2. Broxmeyer, H. E., J. Exp. Med., **183**, 2411 (1996).
3. Fossiez, F., et al., J. Exp. Med., **183**, 2593 (1996).
4. Yao, Z., et al., J. Immunol., **155**, 5483 (1995).
5. Yao, Z., et al., Immunity, **3**, 811 (1995).
6. Kennedy, J., et al., J. Interferon Cytokine Res., **16**, 611 (1996)

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