Human IL-17/IL-17A Antibody
Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-317-NA

DESCRIPTION
Species Reactivity  Human
Specificity  Detects human IL-17 in direct ELISAs and Western blots. In direct ELISAs, approximately 30% cross-reactivity with recombinant canine IL-17 is observed; approximately 10% cross-reactivity with recombinant human IL-17F is observed and less than 5% cross-reactivity with recombinant mouse IL-17 is observed.
Source  Polyclonal Goat IgG
Purification  Antigen Affinity-purified
Immunogen  E. coli-derived recombinant human IL-17
Il620-Ala155
Accession # Q16552
Endotoxin Level  <0.10 EU per 1 µg of the antibody by the LAL method.
Formulation  Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.
*Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS
Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

<table>
<thead>
<tr>
<th>Recommended Concentration</th>
<th>Sample</th>
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<tbody>
<tr>
<td>Western Blot</td>
<td>1 µg/mL See Below</td>
</tr>
<tr>
<td>Immunocytochemistry</td>
<td>5-15 µg/mL Immersion fixed human peripheral blood mononuclear cells treated with PHA</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>1-15 µg/mL See Below</td>
</tr>
<tr>
<td>Immunoprecipitation</td>
<td>3 µg/100 µg cell lysate See Below</td>
</tr>
<tr>
<td>Intracellular Staining by Flow Cytometry</td>
<td>2.5 µg/10⁶ cells See Below</td>
</tr>
<tr>
<td>CyTOF-ready</td>
<td>Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.</td>
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</tbody>
</table>

Neutralization  Measured by its ability to neutralize IL-17-induced IL-6 secretion in NIH/3T3 mouse embryonic fibroblasts. Yao, Z. et al. (1995) Immunity 3:811. The Neutralization Dose (ND⁵₀) is typically 0.02-0.12 µg/mL in the presence of 15 ng/mL Recombinant Human IL-17.

DATA

Western Blot
Detection of Human IL-17 by Western Blot.
Western blot shows lysates of human primary naïve CD4⁺ T cells, along with whole cell lysates (WCL) and conditioned-media supernatant (Supe) of human primary differentiated Th17 cells. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human IL-17 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-317-NA) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for IL-17 at approximately 15 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry
IL-17/IL-17A in Human Tonsil. IL-17/IL-17A was detected in immersion fixed paraffin-embedded sections of human tonsil using Goat Anti-Human IL-17/IL-17A Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-317-NA) at 1 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to lymphocytes. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

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**Immunoprecipitation**

Immunoprecipitation of Human IL-17.

Human IL-17 was immunoprecipitated from 100 μg of human primary differentiated Th17 cell lysate following incubation with 3 μg Goat Anti-Human IL-17 Antibody (Catalog # AF-317-NA) or control antibody (Catalog # AB-108-C) overnight at 4 °C. IL-17-antibody complexes were absorbed using Protein G Sepharose. Immunoprecipitated IL-17 was detected by Western blot using 1 μg/mL Goat Anti-Human IL-17 Antibody (Catalog # AF-317-NA). View our recommended buffer recipes for immunoprecipitation.

**Neutralization**

IL-6 Secretion Induced by IL-17 and Neutralization by Human IL-17 Antibody.

Recombinant Human IL-17 (Catalog # 317-ILB) stimulates IL-6 secretion in NIH/3T3 mouse embryonic fibroblasts in a dose-dependent manner (orange line), as measured by the mouse IL-6 Quantikine ELISA Kit (Catalog # M6000B). IL-6 secretion elicited by Recombinant Human IL-17 (15 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human IL-17 Antibody (Catalog # AF-317-NA). The ND50 is typically 0.02-0.12 μg/mL.

**Intracellular Staining by Flow Cytometry**

Detection of IL-17 in Human PBMCs by Flow Cytometry.

Human peripheral blood mononuclear cells were unstimulated (light orange filled histogram) or treated with 50 ng/mL PMA and 250 ng/mL Ca²⁺ ionomycin for 16 hours, then stained with Goat Anti-Human IL-17 Antibody (Catalog # AF-317-NA, dark orange filled histogram) or isotype control antibody (Catalog # AB-108-C, open histogram), followed by Allophycocyanin-conjugated Anti-Goat IgG Secondary Antibody (Catalog # F0108). To facilitate intracellular staining, cells were fixed with paraformaldehyde and permeabilized with saponin.

**Preparation and Storage**

**Reconstitution**

Reconstitute at 0.2 mg/mL in sterile PBS.

**Shipping**

The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

**Stability & Storage**

- Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.

**Background**

Interleukin 17 (also known as CTLA-8) is a T cell-expressed pleiotropic cytokine that exhibits a high degree of homology to a protein encoded by the ORF13 gene of herpesvirus Saimiri. cDNA clones encoding IL-17 have been isolated from activated rat, mouse and human T cells. Human IL-17 cDNA encodes a 155 amino acid (aa) residue precursor protein with a 19 amino acid residue signal peptide that is cleaved to yield the 136 aa residue mature IL-17 containing one potential N-linked glycosylation site. Both recombinant and natural IL-17 have been shown to exist as disulfide linked homodimers. At the amino acid level, human IL-17 shows 72% and 63% sequence identity with herpesvirus and rat IL-17, respectively. An IL-17 specific mouse cell surface receptor (IL-17 R) has recently been cloned. While the expression of IL-17 mRNA is restricted to activated T cells, the expression of mIL-17 R mRNA has been detected in virtually all cells and tissues tested. IL-17 exhibits multiple biological activities on a variety of cells including the induction of IL-6 and IL-8 production in fibroblasts, the enhancement of surface expression of ICAM-1 in fibroblasts, activation of NF-κB and costimulation of T cell proliferation.