Mouse IL-1β/IL-1F2 Antibody
Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-401-NA

DESCRIPTION
Species Reactivity  Mouse
Specificity  Detects mouse IL-1β/IL-1F2 in direct ELISAs and Western blots. In direct ELISAs, less than 15% cross-reactivity with recombinant porcine IL-1β is observed.
Source  Polyclonal Goat IgG
Purification  Antigen Affinity-purified
Immunogen  E. coli-derived recombinant mouse IL-1β/IL-1F2
           Val118-Ser269
           Accession # NP_032387
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.

Recommended Concentration

<table>
<thead>
<tr>
<th>Application</th>
<th>Sample</th>
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<tbody>
<tr>
<td>Western Blot</td>
<td>0.25 μg/mL</td>
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<tr>
<td>Immunocytochemistry</td>
<td>5-15 μg/mL</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>5-15 μg/mL</td>
</tr>
<tr>
<td>Simple Western</td>
<td>2.5 μg/mL</td>
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</tbody>
</table>

Neutralization
Measured by its ability to neutralize IL-1β/IL-1F2-induced proliferation in the D10.G4.1 mouse helper T cell line. Symons, J.A. et al. (1987) in Lymphokines and Interferons, a Practical Approach. Clemens, M.J. et al. (eds): IRL Press. 272. The Neutralization Dose (ND₅₀) is typically ≤0.25 μg/mL in the presence of 50 pg/mL Recombinant Mouse IL-1β/IL-1F2.

DATA

Western Blot
Detection of Human and Mouse IL-1β/IL-1F2 by Western Blot. Western blot shows lysates of THP-1 human acute monocytic leukemia cell line untreated (-) or treated (+) with 200 nM PMA for 24 hours and 10 μg/mL LPS for 4 hours and RAW 264.7 mouse monocyte/macrophage cell line untreated (-) or treated (+) with 10 μg/mL LPS for 24 hours. PVDF membrane was probed with 0.25 μg/mL of Goat Anti-Mouse IL-1β/IL-1F2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-401-NA) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for IL-1β/IL-1F2 at approximately 35 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunocytochemistry
IL-1β/IL-1F2 in MCF-7 Human Cell Line. IL-1β/IL-1F2 was detected in immersion fixed MCF-7 human breast cancer cell line using Goat Anti-Mouse IL-1β/IL-1F2 Antibody (Catalog # AF-401-NA) at 8 μg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # NL001) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunohistochemistry
IL-1β/IL-1F2 in Mouse Thymus. IL-1β/IL-1F2 was detected in perfusion fixed frozen sections of mouse thymus using Goat Anti-Mouse IL-1β/IL-1F2 Antibody (Catalog # AF-401-NA) at 15 μg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Simple Western
Detection of Mouse IL-1β/IL-1F2 by Simple Western™. Simple Western lane view shows lysates of RAW 264.7 mouse monocyte/macrophage cell line untreated (-) or treated (+) with 10 μg/mL LPS for 24 hours, loaded at 0.5 mg/mL. A specific band was detected for IL-1β/IL-1F2 at approximately 40 kDa (as indicated) using 2.5 μg/mL of Goat Anti-Mouse IL-1β/IL-1F2 Antibody (Catalog # AF-401-NA) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.
Neutralization

Cell Proliferation Induced by IL-1β/IL-1F2 and Neutralization by Mouse IL-1β/IL-1F2 Antibody.

Recombinant Mouse IL-1β/IL-1F2 (Catalog # ML401) stimulates proliferation in the D10.G4.1 mouse helper T cell line in a dose-dependent manner (orange line) as measured by Resazurin (Catalog # AR002). Proliferation elicited by Recombinant Mouse IL-1β/IL-1F2 (50 pg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Mouse IL-1β/IL-1F2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-401-NA). The ND₅₀ is typically ≤0.25 µg/mL.

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

IL-1 is a name that designates two pleiotropic cytokines, IL-1α (IL-1F1) and IL-1β (IL-1F2), which are the products of distinct genes. IL-1α and IL-1β are structurally related polypeptides that share approximately 17% amino acid (aa) identity in mouse. Both proteins are produced by a wide variety of cells in response to inflammatory agents, infections, or microbial endotoxins. While IL-1α and IL-1β are regulated independently, they bind to the same receptor and exert identical biological effects. IL-1 RI binds directly to IL-1α or IL-1β and then associates with IL-1 R accessory protein (IL-1 R3/IL-1 R AcP) to form a high-affinity receptor complex that is competent for signal transduction. IL-1 RI has high affinity for IL-1β but functions as a decoy receptor and negative regulator of IL-1β activity. IL-1ra functions as a competitive antagonist by preventing IL-1α and IL-1β from interacting with IL-1 RI. The mouse IL-1β cDNA encodes a 269 aa precursor. A 17 kDa mature mouse IL-1β shares 90% aa sequence identity with cotton rat and rat and 65-78% identity with canine, equine, feline, human, porcine, and rhesus macaque IL-1β.