Human IL-1ra/IL-1F3 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-280-NA

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human IL-1ra/IL-1F3 in direct ELISAs and Western blots. In direct ELISAs and Western blots (non-reducing conditions), less than 15% cross-reactivity with recombinant mouse IL-1ra is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human IL-1ra/IL-1F3 Arg26-Glu177 Accession # P18510
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.

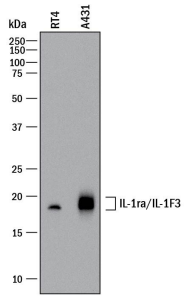
APPLICATIONS

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	Sample
Western Blot	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	20 µg/mL	See Below
Neutralization	Measured by its ability to neutralize IL-1ra/IL-1F3 inhibition of IL-1α/IL-1F1-dependent 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 (ND_{50}) is typically ≤4 µg/mL in the presence of 50 ng/mL Recombinant Human IL-1ra/IL-1F3 and 50 pg/mL Recombinant Human IL-1α/IL-1F1.	

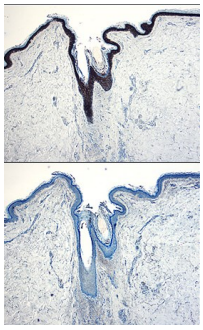
DATA

Western Blot



Detection of Human IL-1ra/IL-1F3 by Western Blot. Western blot shows lysates of RT-4 human bladder carcinoma cell line and A431 human epithelial carcinoma cell line. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human IL-1ra/IL-1F3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-280-NA) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for IL-1ra/IL-1F3 at approximately 18-20 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



IL-1ra/IL-1F3 in Human Skin. IL-1ra/IL-1F3 was detected in immersion fixed paraffin-embedded sections of human skin using Goat Anti-Human IL-1ra/IL-1F3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-280-NA) at 5 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). Specific staining was localized to keratinocytes. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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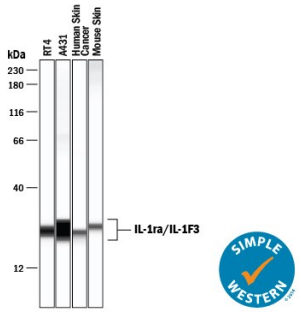
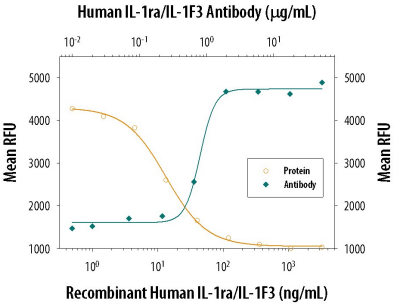
Simple Western



Detection of Human and Mouse IL-1ra/IL-1F3 by Simple Western™. Simple Western lane view shows lysates of RT-4 human bladder carcinoma cell line, A431 human epithelial carcinoma cell line, human skin cancer tissue, and mouse skin cancer tissue, loaded at 0.2 mg/mL. A specific band was detected for IL-1ra/IL-1F3 at approximately 24-26 kDa (as indicated) using 20 µg/mL of Goat Anti-Human IL-1ra/IL-1F3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-280-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



IL-1ra/IL-1F3 Inhibition of IL-1α/IL-1F1-dependent Cell Proliferation and Neutralization by Human IL-1ra/IL-1F3 Antibody. Recombinant Human IL-1ra/IL-1F3 (Catalog # 280-RA) inhibits Recombinant Human IL-1α/IL-1F1 (Catalog # 200-LA) induced proliferation in the D10.G4.1 mouse helper T cell line in a dose-dependent manner (orange line), as measured by Resazurin (Catalog #AR002). Inhibition of Recombinant Human IL-1α/IL-1F1 (50 pg/mL) activity elicited by Recombinant Human IL-1ra/IL-1F3 (50 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human IL-1ra/IL-1F3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-280-NA). The ND_{50} is typically ≤4 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-1ra was originally isolated from the urine of patients with monocytic leukemia and has also been purified from adherent monocytes. The naturally occurring, fully glycosylated form has an apparent molecular weight of about 25,000 Daltons. The protein shows 26% amino acid homology to IL-1β and 19% homology to IL-1α. It will compete with either factor for receptor binding, but does not interact with either one. Human IL-1ra will bind to both types of IL-1 receptor (I and II) on human cells, but reportedly will not block binding to the type II receptor on murine pre-B cell lines. The recombinant, non-glycosylated form of IL-1ra blocks binding of IL-1 to its receptor equally as well as the naturally-occurring, glycosylated form. The IL-1ra has been shown to block the inflammatory responses induced by IL-1 both *in vitro* and *in vivo*. Currently, pre-clinical and clinical studies are underway to test possible therapeutic applications for IL-1ra in the treatment of sepsis, rheumatoid arthritis and chronic myelogenous leukemia.

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