Human IL-18/IL-1F4 Propeptide Antibody

Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF646

DESCRIPTION

Species Reactivity	Human
Specificity	Detects the pro region of human IL-18/IL-1F4 in direct ELISAs and Western blots. In these formats, approximately 5% cross-reactivity with mature recombinant human IL-18 is observed and less than 1% cross-reactivity with mature recombinant mouse IL-18 and recombinant rat IL-18 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human IL-18/IL-1F4
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.

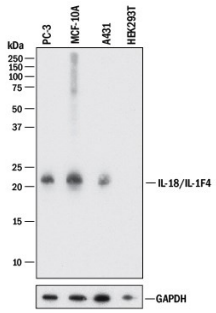
APPLICATIONS

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
Simple Western	50 µg/mL	See Below
Knockout Validated	IL-18/IL-1F4 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in IL-18/IL-1F4 knockout HeLa cell line.	

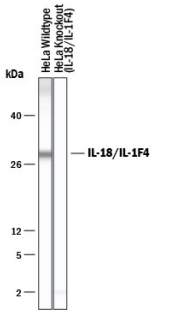
DATA

Western Blot



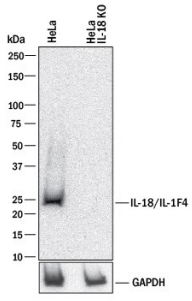
Detection of Human IL-18/IL-1F4 by Western Blot. Western blot shows lysates of PC-3 human prostate cancer cell line, MCF 10A human breast epithelial cell line, A431 human epithelial carcinoma cell line, and HEK293T human embryonic kidney cell line (negative control cell line). PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human IL-18/IL-1F4 Propeptide Antigen Affinity-purified Polyclonal Antibody (Catalog # AF646) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for IL-18/IL-1F4 at approximately 22 kDa (as indicated). GAPDH (Catalog # Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Simple Western



Detection of Human IL-18/IL-1F4 by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma parental cell line and IL-18/IL-1F4 knockout HeLa cell line, loaded at 0.2 mg/mL. A specific band was detected for IL-18/IL-1F4 at approximately 29 kDa (as indicated) in the HeLa parental cell line using 50 µg/mL of Goat Anti-Human IL-18/IL-1F4 Propeptide Antigen Affinity-purified Polyclonal Antibody (Catalog # AF646) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

Knockout Validated



Western Blot Shows Human IL-18/IL-1F4 Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and IL-18/IL-1F4 knockout HeLa cell line (KO). PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human IL-18/IL-1F4 Propeptide Antigen Affinity-purified Polyclonal Antibody (Catalog # AF646) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for IL-18/IL-1F4 at approximately 22 kDa (as indicated) in the parental HeLac ell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

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Human IL-18/IL-1F4 Propeptide Antibody

Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF646

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

Pro-IL-18 (pro-Interleukin 18; also pro-IGIF and pro-IL-1γ) is a 24 kDa member of the IL-1 family of molecules. It is widely expressed, being produced by keratinocytes, intestinal epithelium, T cells, macrophages and osteoblasts. Human Pro-IL-18 is 193 amino acids (aa) in length. Although mature IL-18 induces IFN-γ secretion by NK and T cells, Pro-IL-18 appears to have little intrinsic activity. Generally, active IL-18 is considered to arise from caspase-1 cleavage of Pro-IL-18 between Asp36-Tyr37. This generates an 18 kDa mature C-terminal fragment, and a 4 kDa (predicted) N-terminal prosegment that runs at 6 kDa in SDS-PAGE. Other proteases are known to process Pro-IL-18. Caspase-3 cleavage after Asp68 generates an inactive 14 kDa mature segment, Merpin β-subunit cleavage after Asn52 generates a marginally active 17 kDa mature segment, while parasite Cys protease cleavage after Val47 generates an inactive 17 kDa mature molecule. One splice variant shows a deletion of aa 27-30. Over aa 2-36, human Pro-IL-18 shares 63% aa identity with mouse Pro-IL-18.

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