

### DESCRIPTION

<b>Species Reactivity</b>	Human/Rhesus Macaque
<b>Specificity</b>	Detects rhesus macaque IL-18/IL-1F4 in direct ELISAs and Western blots. In Western blots, approximately 10% cross-reactivity with recombinant mouse IL-18, recombinant rat IL-18, and recombinant porcine IL-18 is observed.
<b>Source</b>	Polyclonal Goat IgG
<b>Purification</b>	Antigen Affinity-purified
<b>Immunogen</b>	<i>E. coli</i> -derived recombinant rhesus macaque IL-18/IL-1F4 Tyr37-Asp193 Accession # AAK13416
<b>Endotoxin Level</b>	<0.10 EU per 1 µg of the antibody by the LAL method.
<b>Formulation</b>	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.

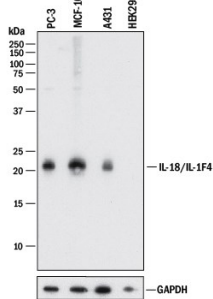
### APPLICATIONS

**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
<b>Western Blot</b>	0.5 µg/mL	See Below
<b>Immunocytochemistry</b>	5-15 µg/mL	See Below
<b>Simple Western</b>	50 µg/mL	See Below
<b>Knockout Validated</b>	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.	
<b>Neutralization</b>	Measured by its ability to neutralize IL-18/IL-1F4-induced IFN-γ secretion in the KG-1 human acute myelogenous leukemia cell line. Novick, D. <i>et al.</i> (1999) <i>Immunity</i> <b>10</b> (1):127. The Neutralization Dose (ND <sub>50</sub> ) is typically 0.4-1.2 µg/mL in the presence of 10 ng/mL Recombinant Rhesus Macaque IL-18/IL-1F4 and 20 ng/mL Recombinant Human TNF-α.	
<b>ELISA</b>	This antibody functions as an ELISA detection antibody when paired with Mouse Anti-Human IL-18/IL-1F4 Monoclonal Antibody (Catalog # <a href="#">MAB91242</a> ).  <i>This product is intended for assay development on various assay platforms requiring antibody pairs. We recommend the Human Total IL-18 DuoSet ELISA Kit (Catalog # <a href="#">DY318-05</a>) for convenient development of a sandwich ELISA or the Human Total IL-18/IL-1F4 Quantikine ELISA Kit (Catalog # <a href="#">DL180</a>) for a complete optimized ELISA.</i>	

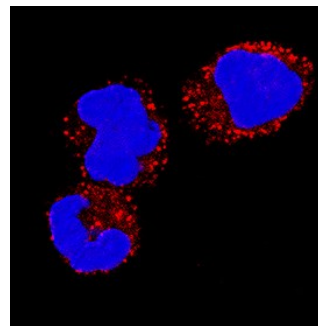
### DATA

#### Western Blot



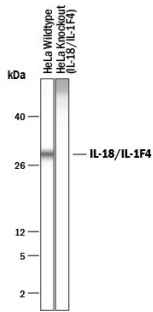
**Detection of Human IL-18/IL-1F4 by Western Blot.** Western blot shows lysates of PC-3 human prostate cancer cell line, MCF 10A human breast epithelial cell line, A431 human epithelial carcinoma cell line, and HEK293T human embryonic kidney cell line (negative control cell line). PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Rhesus Macaque IL-18/IL-1F4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2548) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # [HAF017](#)). A specific band was detected for IL-18/IL-1F4 at approximately 22 kDa (as indicated). GAPDH (Catalog # [AF5718](#)) is shown as a loading control. This experiment was conducted under reducing conditions and using [Immunoblot Buffer Group 1](#).

#### Immunocytochemistry



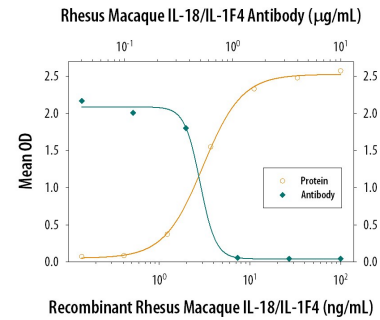
**IL-18/IL-1F4 in Human PBMCs.** IL-18/IL-1F4 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) using Goat Anti-Human/Rhesus Macaque IL-18/IL-1F4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2548) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # [NL001](#)) and counterstained with DAPI (green). Specific staining was localized to cytoplasm. View our protocol for [Fluorescent ICC Staining of Non-adherent Cells](#).

### Simple Western



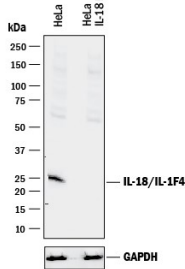
**Detection of Human IL-18/IL-1F4 by Simple Western™.** Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma parental cell line and IL-18/IL-1F4 knockout HeLa cell line, loaded at 0.2 mg/mL. A specific band was detected for IL-18/IL-1F4 at approximately 29 kDa (as indicated) in the HeLa parental cell line using 50 µg/mL of Goat Anti-Human/Rhesus Macaque IL-18/IL-1F4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2548) 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 2-40 kDa separation system.

### Neutralization



**IFN-γ Secretion Induced by IL-18/IL-1F4 and Neutralization by Primate IL-18/IL-1F4 Antibody.** In the presence of Recombinant Human TNF-α (20 ng/mL, Catalog # Catalog # 210-TA), Recombinant Rhesus Macaque IL-18/IL-1F4 (Catalog # Catalog # 2548-RM) stimulates IFN-γ secretion in the KG-1 human acute myelogenous leukemia cell line in a dose-dependent manner (orange line), as measured by the Human IFN-γ Quantikine ELISA Kit (Catalog # Catalog # DIF50C). Under these conditions, IFN-γ secretion elicited by Recombinant Rhesus Macaque IL-18/IL-1F4 (10 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human/Rhesus Macaque IL-18/IL-1F4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2548). The ND<sub>50</sub> is typically 0.4-1.2 µg/mL.

### 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 1 µg/mL of Goat Anti-Human/Rhesus Macaque IL-18/IL-1F4 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF2548) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for IL-18/IL-1F4 at approximately 25 kDa (as indicated) in the parental HeLa cell 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.

### PREPARATION AND STORAGE

<b>Reconstitution</b>	Reconstitute at 0.2 mg/mL in sterile PBS.
<b>Shipping</b>	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
<b>Stability &amp; Storage</b>	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. <ul style="list-style-type: none"> <li>• 12 months from date of receipt, -20 to -70 °C as supplied.</li> <li>• 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li> <li>• 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li> </ul>

## BACKGROUND

Interleukin-18 (IL-18), also known as IL-1F4 and IFN- $\gamma$  inducing factor (IGIF), is a member of the IL-1 family of cytokines and is a key molecule in the innate immune response (1). Rhesus IL-18 is synthesized as a 24 kDa proprotein that contains a 36 amino acid (aa) propeptide and a 157 aa mature region (2). Under inflammatory conditions, the propeptide is cleaved by Caspase-1 in the cytoplasm to liberate the mature nonglycosylated 18 kDa monomeric IL-18 (3, 4). Mature rhesus IL-18 shares 96% aa sequence identity with human IL-18 and 60-76% with mouse, rat, canine, feline, and porcine IL-18. IL-18 is secreted by a variety of cell types including macrophages, dendritic cells, and epithelial cells (1, 5). Circulating mature IL-18 is sequestered by soluble IL-18 binding proteins (IL-18 BP) that inhibit IL-18 bioactivity (6). IL-18 interacts with the widely expressed IL-18 R $\alpha$  which then recruits the signaling subunit IL-18 R $\beta$  (7, 8). The IL-1 family member IL-1F7 also binds to IL-18 R $\alpha$  but does not recruit IL-18 R $\beta$  or induce signaling (9). IL-1F7 binds IL-18 BP and enhances its neutralizing effect on IL-18 activity (9). IL-18 synergizes with other cytokines to activate NK, Th1, and Th17 cells and to increase the production of IFN- $\gamma$  (1, 5, 10-12). IL-18 can also promote Th2 cytokine release which reduces the effectiveness of antiviral responses (13, 14). Increased levels of active IL-18 contribute to the severity of autoimmunity and hypertension, while deficiency of IL-18 results in symptoms of metabolic syndrome (1, 5, 15, 16). In cancer, IL-18 stimulates Th1 and NK cells to target tumor cells, but it can also promote angiogenesis, metastasis, and tumor cell immune evasion (11).

## References:

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