Human IL-8/CXCL8 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF-208-NA

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human IL-8/CXCL8 in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human IL-8/CXCL8 Ser28-Ser99 Accession # P10145
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.

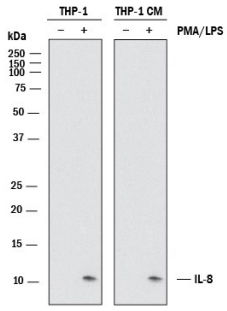
APPLICATIONS

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	2 µg/mL	THP-1 human acute monocytic leukemia cell line treated (+) with PMA and LPS
Immunocytochemistry	5-15 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	25 µg/mL	THP-1 human acute monocytic leukemia cell line treated with PMA and LPS and PBMC continioned media treated with LPS
Neutralization	Measured by its ability to neutralize IL-8/CXCL8-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CXCR2. The Neutralization Dose (ND_{50}) is typically 0.1-0.5 µg/mL in the presence of 20 ng/mL Recombinant Human IL-8/CXCL8.	

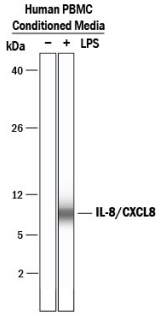
DATA

Western Blot



Detection of Human IL-8/CXCL8 by Western Blot. Western blot shows lysates and conditioned media of THP-1 human acute monocytic leukemia cell line untreated (-) or treated (+) with 200 nM PMA and 10 ug/mL LPS for 24 hours and 3 hours. PVDF membrane was probed with 2 µg/mL of Goat Anti-Human IL-8/CXCL8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-208-NA) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for IL-8/CXCL8 at approximately 10 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Simple Western



Detection of Human IL-8/CXCL8 by Simple Western™. Simple Western lane view shows PBMC conditioned media untreated (-) or treated (+) with 1 µg/mL LPS for 24 hrs, loaded at 0.2 mg/mL. A specific band was detected for IL-8/CXCL8 at approximately 8 kDa (as indicated) using 25 µg/mL of Goat Anti-Human IL-8/CXCL8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-208-NA) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). This experiment was conducted under reducing conditions and using the 2-40 kDa separation system.

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Antigen Affinity-purified Polyclonal Goat IgG
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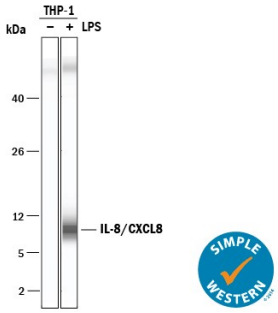
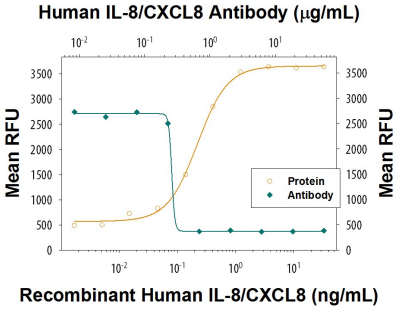
Simple Western



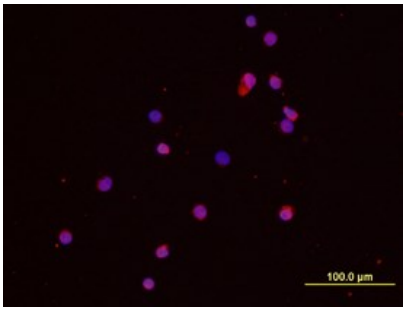
Detection of Human IL-8/CXCL8 by Simple Western™. Simple Western lane view shows lysates of THP-1 human acute monocytic leukemia cell line untreated (-) or treated (+) with 200 nM PMA for 24 hrs and 10ug/mL LPS for 3 hrs, loaded at 0.2 mg/mL. A specific band was detected for IL-8/CXCL8 at approximately 8 kDa (as indicated) using 25 µg/mL of Goat Anti-Human IL-8/CXCL8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-208-NA) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). This experiment was conducted under reducing conditions and using the 2-40 kDa separation system.

Neutralization



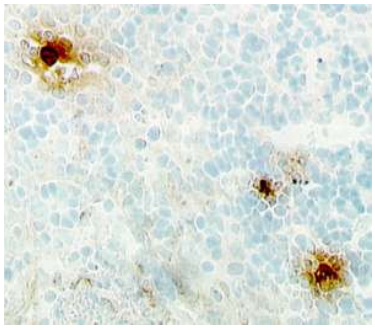
Chemotaxis Induced by IL-8/CXCL8 and Neutralization by Human IL-8/CXCL8 Antibody. Recombinant Human IL-8/CXCL8 (Catalog # Catalog # 208-IL) chemoattracts the BaF3 mouse pro-B cell line transfected with human CXCR2 in a dose-dependent manner (orange line). The amount of cells that migrated through to the lower chemotaxis chamber was measured by Resazurin (Catalog # Catalog # AR002). Chemotaxis elicited by Recombinant Human IL-8/CXCL8 (20 ng/mL) is neutralized (green line) by increasing concentrations of Human IL-8/CXCL8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-208-NA). The ND_{50} is typically 0.1-0.5 µg/mL.

Immunocytochemistry



IL-8/CXCL8 in Human PBMCs. IL-8/CXCL8 was detected in immersion fixed human peripheral blood mononuclear cells (PBMCs) stimulated with PMA and ionomycin using 10 µg/mL Human IL-8/CXCL8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-208-NA) for 3 hours at room temperature. Cells were stained with the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red; Catalog # Catalog # NL001) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Non-adherent Cells.

Immunohistochemistry



IL-8/CXCL8 in Human Tonsil. IL-8/CXCL8 was detected in immersion fixed paraffin-embedded sections of human tonsil (surgically removed due to severe EBV-induced mononucleosis) using Human IL-8/CXCL8 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF-208-NA) overnight at 4 °C. Tissue was stained (brown) and counterstained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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

CXCL8 was originally discovered and purified independently by a number of laboratories as a neutrophil chemotactic and activating factor. It was also referred to as neutrophil chemotactic factor (NCF), neutrophil activating protein (NAP), monocyte-derived neutrophil chemotactic factor (MDNCF), T-lymphocyte chemotactic factor (TCF), granulocyte chemotactic protein (GCP) and leukocyte adhesion inhibitor (LAI). Many cell types, including monocyte/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, keratinocytes, hepatocytes, chondrocytes, and various tumor cell lines, can produce CXCL8 in response to a wide variety of pro-inflammatory stimuli such as exposure to IL-1, TNF, LPS, and viruses. CXCL8 is a member of the alpha (CXC) subfamily of chemokines, which also includes platelet factor 4, GRO, IP-10, etc.

CXCL8 is a potent chemoattractant for neutrophils. In addition, CXCL8 also has a wide range of other pro-inflammatory effects. CXCL8 causes degranulation of neutrophil specific granules and azurophilic granules. CXCL8 induces expression of the cell adhesion molecules CD11/CD18 and enhances the adherence of neutrophils to endothelial cells and sub-endothelial matrix proteins. Besides neutrophils, CXCL8 is also chemotactic for basophils, T cells and eosinophils. CXCL8 has been reported to be a co-mitogen for keratinocytes and was also shown to be an autocrine growth factor for melanoma cells. CXCL8 was also reported to be angiogenic both *in vivo* and *in vitro*.

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