Human CXCL5/ENA-78 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF254

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human CXCL5/ENA-78 in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	*E. coli*-derived recombinant human CXCL5/ENA-78 Ala37-Asn114 Accession # P42830
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.

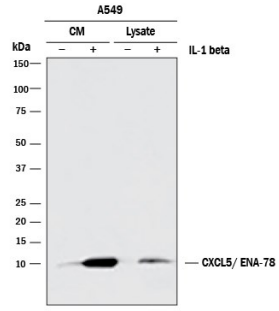
APPLICATIONS

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	1 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Neutralization	Measured by its ability to neutralize CXCL5/ENA-78-induced chemotaxis in the BaF3 mouse pro-B cell line transfected with human CXCR2. The Neutralization Dose (ND_{50}) is typically 0.2-1.0 µg/mL in the presence of 0.03 µg/mL Recombinant Human CXCL5/ENA-78.	

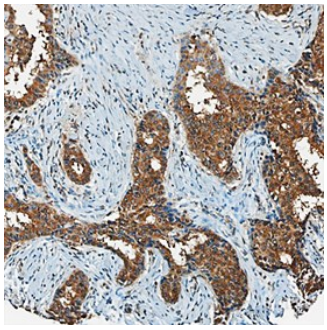
DATA

Western Blot



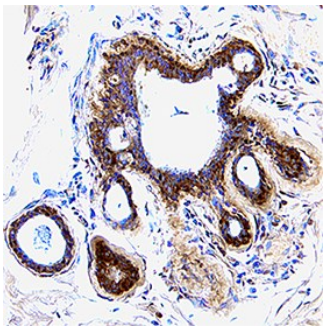
Detection of Human CXCL5/ENA-78 by Western Blot. Western blot shows conditioned media (CM) and lysates from A549 human lung carcinoma cell line untreated (-) or treated (+) with human IL-1 beta for 24 hours. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human CXCL5/ENA-78 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF254) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). A specific band was detected for CXCL5/ENA-78 at approximately 10 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



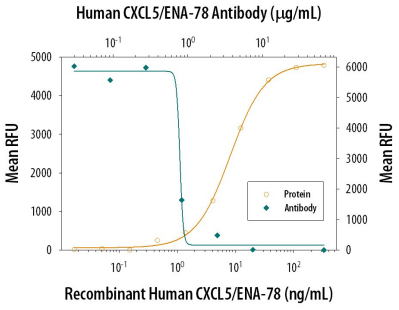
CXCL5/ENA-78 in Human Breast Cancer Tissue. CXCL5/ENA-78 was detected in immersion fixed paraffin-embedded sections of human breast cancer tissue using Goat Anti-Human CXCL5/ENA-78 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF254) at 10 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; (Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Immunohistochemistry



CXCL5/ENA-78 in Human Breast. CXCL5/ENA-78 was detected in immersion fixed paraffin-embedded sections of human breast using Goat Anti-Human CXCL5/ENA-78 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF254) 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 epithelial cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Neutralization



Chemotaxis Induced by CXCL5/ENA-78 and Neutralization by Human CXCL5/ENA-78 Antibody. Recombinant Human CXCL5/ENA-78 (Catalog # 254-XB) 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 # AR002). Chemotaxis elicited by Recombinant Human CXCL5/ENA-78 (0.03 µg/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human CXCL5/ENA-78 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF254). The ND_{50} is typically 0.2-1.0 µg/mL.

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Antigen Affinity-purified Polyclonal Goat IgG

Catalog Number: AF254

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

CXCL5, also known as epithelial cell-derived neutrophil-activating peptide (ENA-78), is an 8 kDa proinflammatory member of the CXC subfamily of chemokines. Its Glu-Leu-Arg (ELR) motif confers angiogenic properties and distinguishes it from ELR-CXC chemokines which are angiostatic (1-3). Human CXCL5 shares 57% amino acid (aa) sequence identity with mouse and rat CXCL5. Among other human ELR+ chemokines, it shares 77% aa sequence identity with CXCL6/GCP-2 and 35%-51% with CXCL1/GRO alpha, CXCL2/GRO beta, CXCL3/GRO gamma, CXCL7/NAP-2, and CXCL8/IL-8. Inflammatory stimulation up-regulates CXCL5 production in multiple hematopoietic cell types, fibroblasts, endothelial cells, and vascular smooth muscle cells. *In vivo*, CXCL5 is elevated at sites of inflammation and pulmonary fibrosis where it promotes neutrophil infiltration and activation as well as angiogenesis (3-6). Its upregulation contributes to increased vascularization, tumor growth, and metastasis in many cancers (6-9). Full length CXCL5 (78 aa) is trimmed at the N-terminus by cathepsin G and chymotrypsin to ENA-74 (74 aa) and ENA-70 (70 aa), with the shortened forms showing increased potency relative to full length CXCL5 (10, 11). CXCL5 exerts its effects primarily through interactions with CXCR2 (6, 12). It also binds duffy antigen receptor for chemokines (DARC), which can limit CXCR2-mediated responses (13, 14).

References:

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