Human TIMP-1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF970

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human TIMP-1 in direct ELISAs and Western blots. In direct ELISAs, approximately 20% cross-reactivity with recombinant mouse TIMP-1 and recombinant rat TIMP-1 is observed and less than 1% cross-reactivity with recombinant human (rh) TIMP-2, rhTIMP-3, and rhTIMP-4 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human TIMP-1 Cys24-Ala207 Accession # Q6FGX5
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.

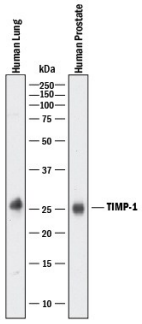
APPLICATIONS

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	1-5 µg/mL	See Below
Immunohistochemistry	1-15 µg/mL	See Below
Simple Western	50 µg/mL	See Below
Knockout Validated	TIMP-1 is specifically detected in SK-OV-3 human ovarian adenocarcinoma parental cell line but is not detectable in TIMP-1 knockout SK-OV-3 cell line.	
Neutralization	Measured by its ability to neutralize Recombinant Human TIMP-1 (0.1 µg/mL, Catalog # 970-TM) inhibition of Recombinant Human MMP-2 (0.2 µg/mL, Catalog # 902-MP) cleavage of the fluorogenic peptide substrate Mca-PLGL-Dpa-AR-NH_2 (5 µM, Catalog # ES001). The Neutralization Dose (ND_{50}) is typically 1 µg/mL.	

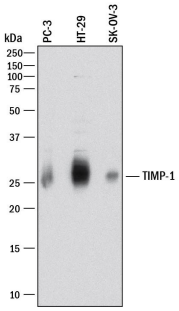
DATA

Western Blot



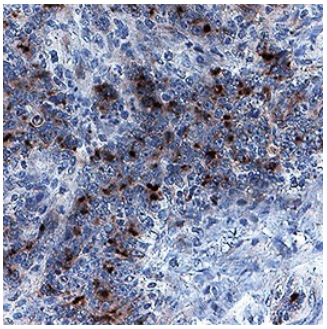
Detection of Human TIMP-1 by Western Blot. Western blot shows lysates of human lung tissue and human prostate tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human TIMP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF970) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for TIMP-1 at approximately 25 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



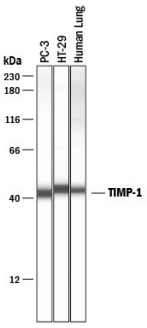
Detection of Human TIMP-1 by Western Blot. Western blot shows lysates of PC-3 human prostate cancer cell line, HT-29 human colon adenocarcinoma cell line, and SK-OV-3 human ovarian adenocarcinoma cell line. PVDF membrane was probed with 5 µg/mL of Goat Anti-Human TIMP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF970) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for TIMP-1 at approximately 26 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



TIMP-1 in Human Colon Cancer Tissue. TIMP-1 was detected in immersion fixed paraffin-embedded sections of human colon cancer tissue using Goat Anti-Human TIMP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF970) at 1 µg/mL for 1 hour at room temperature followed by incubation with the Anti-Goat IgG VisUCyte™ HRP Polymer Antibody (Catalog # VC004). Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using DAB (brown) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm and extracellular space. View our protocol for IHC Staining with VisUCyte HRP Polymer Detection Reagents.

Simple Western



Detection of Human TIMP-1 by Simple Western™. Simple Western lane view shows lysates of PC-3 human prostate cancer cell line, HT-29 human colon adenocarcinoma cell line, and human lung tissue, loaded at 0.2 mg/mL. A specific band was detected for TIMP-1 at approximately 42-45 kDa (as indicated) using 50 µg/mL of Goat Anti-Human TIMP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF970) 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.

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Human TIMP-1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
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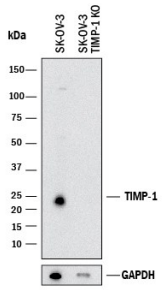
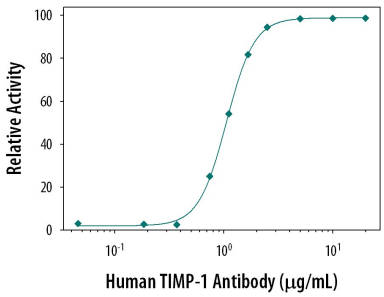
Knockout Validated



Western Blot Shows Human TIMP-1 Specificity by Using Knockout Cell Line. Western blot shows lysates of SK-OV-3 human ovarian adenocarcinoma cell line and TIMP-1 knockout SK-OV-3 cell line (KO). PVDF membrane was probed with 2 µg/mL of Goat Anti-Human TIMP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF970) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for TIMP-1 at approximately 25 kDa (as indicated) in the parental SK-OV-3 cell line, but is not detectable in knockout SK-OV-3 cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Neutralization



Neutralization of TIMP-1 Activity by Human TIMP-1 Antibody. Recombinant Human MMP-2 (0.2 µg/mL, Catalog # 902-MP) activity is measured in the presence of Recombinant Human TIMP-1 (0.1 µg/mL, Catalog # 970-TM) that has been preincubated with increasing concentrations of Goat Anti-Human TIMP-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF970). The ND_{50} is typically 1 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

Tissue inhibitors of metalloproteinases or TIMPs are a family of proteins that regulate the activation and proteolytic activity of the zinc enzymes known as matrix metalloproteinases (MMPs). There are four members of the family, TIMP-1, TIMP-2, TIMP-3 and TIMP-4. TIMP-1 is a glycoprotein with a molecular mass of 28 kDa produced by a wide range of cell types. TIMP-1 inhibits active MMP-mediated proteolysis by forming an N-terminal, non-covalent binary complex with the MMP active site. TIMP-1 also associates C-terminally with Pro-MMP-9 in a complex which may play a role in regulating activation. Independent of MMPs, TIMP-1 has been shown to have a role in tissue homeostasis.

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