Human/Mouse TIMP-2 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF971

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse TIMP-2 in direct ELISAs and Western blots. In direct ELISAs, less than 2% cross-reactivity with recombinant human TIMP-4 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human TIMP-2 Cys27-Pro220 Accession # P16035
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.

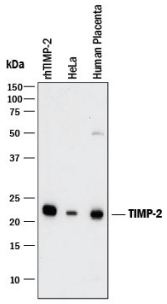
APPLICATIONS

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
Dual RNAscope ISH-IHC Compatible	5-15 µg/mL	Immersion fixed paraffin-embedded sections of human placenta
Western Blot	1 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Knockout Validated	TIMP-2 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in TIMP-2 knockout HeLa human cervical epithelial carcinoma cell line.	
Neutralization	Measured by its ability to neutralize Recombinant Human TIMP-2 (0.143 µg/mL, Catalog # 971-TM) inhibition of Recombinant Human MMP-2 (0.2 µg/mL, Catalog # 902-MPN or 902-MP) cleavage of the fluorogenic peptide substrate Mca-PLGL-Dpa-AR-NH_2 (10 µM, Catalog # ES001). The Neutralization Dose (ND_{50}) is typically 2.6 µg/mL.	

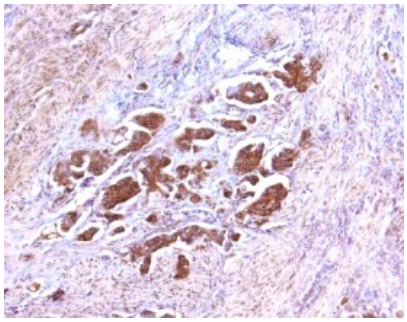
DATA

Western Blot



Detection of Human TIMP-2 by Western Blot. Western blot shows Recombinant Human TIMP-2 Western Blot Standard Protein (Catalog # Catalog # WBC023) and lysates of HeLa human cervical epithelial carcinoma cell line and human placenta tissue. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse TIMP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF971) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF017). A specific band was detected for TIMP-2 at approximately 22 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



TIMP-2 in Human Ovarian Cancer Tissue. TIMP-2 was detected in immersion fixed paraffin-embedded sections of human ovarian cancer tissue using 15 µg/mL Goat Anti-Human/Mouse TIMP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF971) overnight at 4 °C. Tissue was stained with the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # Catalog # CTS008) and counter-stained with hematoxylin (blue). View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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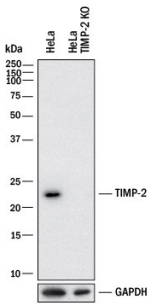
Human/Mouse TIMP-2 Antibody

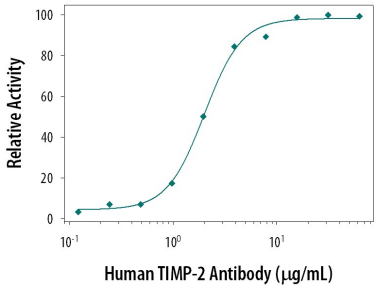
Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF971

Knockout Validated



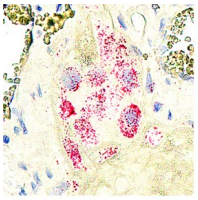
Western Blot Shows Human TIMP-2 Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and human TIMP-2 knockout HeLa human cervical epithelial carcinoma cell line (KO). PVDF membrane was probed with 1 µg/mL of Goat Anti-Human/Mouse TIMP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF971) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for TIMP-2 at approximately 22 kDa (as indicated) in the parental HeLa human cervical epithelial carcinoma cell line, but is not detectable in knockout HeLa human cervical epithelial carcinoma cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Neutralization

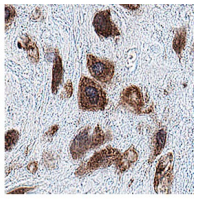


Neutralization of TIMP-2 Activity by Human TIMP-2 Antibody. Recombinant Human MMP-2 (0.2 µg/mL, Catalog # 902-MPNor Catalog # 902-MP) activity is measured in the presence of Recombinant Human/Mouse TIMP-2 (0.143 µg/mL, Catalog # Catalog # 971-TM) that has been preincubated with increasing concentrations of Goat Anti-Human TIMP-2 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF971). The ND_{50} is typically 2.6 µg/mL.

In-situ Hybridization



In Situ Hybridization (ISH)



Immunohistochemistry (IHC)

Detection of TIMP-2 in Human Placenta. Formalin-fixed paraffin-embedded tissue sections of human placenta were probed for TIMP2 mRNA (ACD RNAScope Probe, catalog #470358; Fast Red chromogen, ACD catalog # 322750). Adjacent tissue section was processed for immunohistochemistry using goat anti-human TIMP2 polyclonal antibody (R&D Systems catalog # Catalog # AF971) at 5ug/mL with overnight incubation at 4 degrees Celsius followed by incubation with anti-goat IgG VisUCyte HRP Polymer Antibody (Catalog # Catalog # VC004) and DAB chromogen (yellow-brown). Tissue was counterstained with hematoxylin (blue). Specific staining was localized to decidual cells.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.2 mg/mL in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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-2 is a non N-glycosylated protein with a molecular mass of 22 kDa produced by a wide range of cell types, which inhibits MMPs non-covalently by the formation of binary complexes. TIMP-2 also has erythroid-potentiating and cell growth promoting activities.

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