Human Thrombospondin-1 Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF3074

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Thrombospondin-1 in direct ELISAs and Western blots.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human Thrombospondin-1 Asn19-Pro1170 Accession # CAA32889
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.

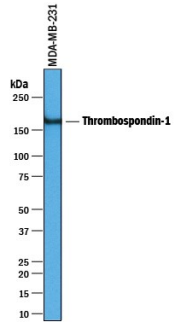
APPLICATIONS

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
Simple Western	10 µg/mL	See Below

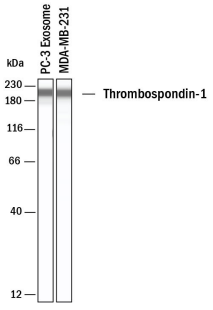
DATA

Western Blot



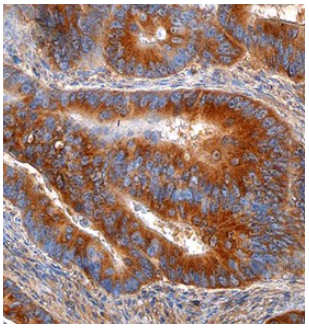
Detection of Human Thrombospondin-1 by Western Blot. Western blot shows lysates of MDA-MB-231 human breast cancer cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human Thrombospondin-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3074) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF019). A specific band was detected for Thrombospondin-1 at approximately 160 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Simple Western



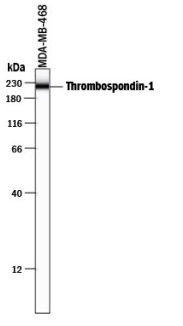
Detection of Human Thrombospondin-1 by Simple Western™. Simple Western lane view shows lysates of Exosome Standards (PC-3) (Catalog # NBP2-49856) and MDA-MB-231 human breast cancer cell line, loaded at 0.5 mg/ml. A specific band was detected for Thrombospondin-1 at approximately 210 kDa (as indicated) using 10 µg/ml of Goat Anti-Human Thrombospondin-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3074) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). This experiment was conducted under reducing conditions and using the 12-230kDa separation system.

Immunohistochemistry



Thrombospondin-1 in Human Colon. Thrombospondin-1 was detected in immersion fixed paraffin-embedded sections of human colon using Goat Anti-Human Thrombospondin-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3074) at 1.7 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to the cytoplasm of epithelial cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western



Detection of Human Thrombospondin-1 by Simple Western™. Simple Western lane view shows lysates of MBA-MB-468 human breast cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for Thrombospondin-1 at approximately 221 kDa (as indicated) using 10 µg/mL of Goat Anti-Human Thrombospondin-1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3074) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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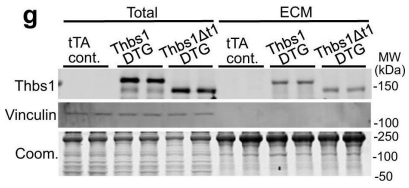
Western Blot



Detection of Mouse Thrombospondin-1 by Western Blot Endothelial cells and TGFβ are not affected by cardiac Thbs1 overexpression.a Quantification of capillary number per mm2 of tissue from histological sections of tTA cont. and Thbs1 DTG hearts stained with isolectin B4 at 6 weeks of age. b Quantification of endothelial cell proliferation as measured by EdU incorporation co-labeled with CD31 in tTA cont. and Thbs1 DTG hearts at 6 weeks of age. c Quantification of endothelial cell apoptosis detected by TUNEL staining co-labeled with isolectin B4 in tTA cont. and Thbs1 DTG hearts at 6 weeks of age. d ELISA-based quantification of total TGFβ and e active TGFβ in protein extracts from tTA cont. or Thbs1 DTG hearts at 6 weeks of age. f Schematic diagram of WT Thbs1 domain structure and the Thbs1Δt1 mutant lacking the Thbs1 type-1 repeat domain region. g Representative western blot analysis for Thbs1 from total protein extracts (Total) and extracellular matrix (ECM) extracts from hearts of tTA cont., Thbs1 DTG, and Thbs1 DTG Δt1 mice at 4 weeks of age. Vinculin is presented as cytosolic control. Coomassie stained (Coom.) gel is shown as loading control. h VW/BW ratio at 4 weeks of age in the indicated groups of mice. *P < 0.0001 versus tTA cont.; statistical analysis was performed using one-way ANOVA and Tukey multiple comparisons test. i Kaplan–Meier survival plot of tTA cont., Thbs1 DTG, and Thbs1Δt1 DTG animals. *P < 0.0001 vs tTA cont. #P < 0.0001 vs Thbs1 DTG; both analyzed by two-tailed log-rank test. The same data from Fig. 2e are shown again here for tTA cont. and Thbs1 DTG mice (same strain and ages and sex ratio mix). j Representative western blots for Thbs2 and Gapdh as loading control, from heart protein extracts from tTA cont. and Thbs2 DTG mice at 8 weeks of age. k Heart weight (HW)/BW ratio, and l FS percentage at 8 weeks of age from tTA cont. and Thbs2 DTG mice. The number of biologically independent animals analyzed is indicated on each graph. Error bars are ±standard error of the mean. Source data are provided as a Source Data File. Image collected and cropped by CiteAb from the following publication (https://pubmed.ncbi.nlm.nih.gov/34168130), licensed under a CC-BY license. Not internally tested by R&D Systems.

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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

Thrombospondin-1 (TSP-1) is a 150-180 kDa charter member of the thrombospondin family of extracellular matrix proteins. Human TSP-1 is synthesized as an 1170 amino acid (aa) precursor that contains an 18 aa signal sequence and 1152 aa mature molecule. The mature molecule has been described as containing three distinct regions that create the shape of a dumbbell. There is an intital, 140 aa N-terminal laminin G-like globular region that binds heparin (aa 19-258). This is followed by an extended, central collagen-binding region that contains one type C von Willebrand factor domain, plus three TSP type I and three TSP type II (or EGF-like) domains (aa 259-712). The C-terminus (aa 713-1170) appears as a large globule with two halves; one calcium-binding region (aa 713-950) with seven Asp-rich TSP type III domains, and one terminal region (aa 951-1170) with TSP-unique motifs (1). This C-terminal region is believed to mediate CD47 and cell binding (2-5). The TSP type I repeats have multiple functions. They bind to type V collagen, laminin, fibronectin and CD36. They also contain a recognition site for C-mannosylation on Trp. Finally, a type I KRFK motif induces the release of mature TGF-b from LAP. This is an effect not found in TSP-2. The function of the type II repeats in unclear. TSP-1 is secreted as a disulfide-linked 450 kDa homotrimer. The cysteines responsible lie just N-terminal to the first type I TSP repeat. Mature human TSP-1 is 61% aa identical to human TSP-2. It is also 95%, 97% and 95% aa identical to mouse, dog and rat TSP-1, respectively.

References:

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