

Product Datasheet

ADAMTS13 Antibody - BSA Free NB100-584

Unit Size: 100 ul

Store at 4C. Do not freeze.

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NB100-584

ADAMTS13 Antibody - BSA Free

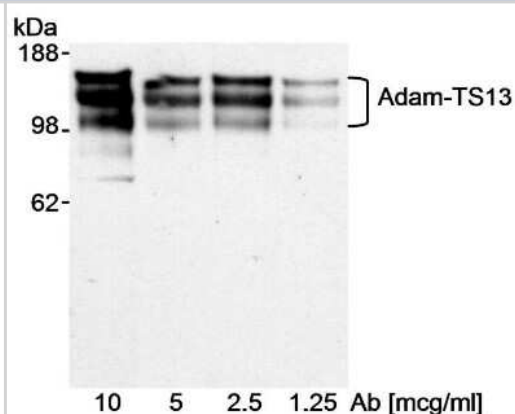
Product Information	
Unit Size	100 ul
Concentration	1.0 mg/ml
Storage	Store at 4C. Do not freeze.
Clonality	Polyclonal
Preservative	0.09% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	Tris-Citrate/Phosphate (pH 7.0 - 8.0)

Product Description	
Host	Goat
Gene ID	11093
Gene Symbol	ADAMTS13
Species	Human
Specificity/Sensitivity	NB100-584 is specific for human ADAM TS13 protein.
Immunogen	A synthetic peptide which maps to a region between residues 250 and 350 of human A Disintegrin-like and Metalloprotease (reprolysin type) with Thrombospondin type 1 motif, 13 using the numbering given in entry XP_088414.1 (GeneID 11093).

Product Application Details	
Applications	Western Blot
Recommended Dilutions	Western Blot 1:100-1:1000
Application Notes	NB 100-584 has been shown to react with purified recombinant ADAM-TS13 in Western Blot. It has not been tested for reactivity with ADAM-TS13 from native sources, and the antibody is not warranted to have such activity. The suggested WB dilution is for recombinant protein.

Images

Western Blot: ADAMTS13 Antibody [NB100-584] - Purified recombinant Adam-TS13. Antibody used at the indicated concentrations.



Publications

Hunt RC, Geetha S, Allen CE et al. Detection of a secreted metalloprotease within the nuclei of liver cells. Mol Biosyst 2011-06-01 [PMID: 21479334]

Chung MC, Popova TG, Jorgensen SC et al. Degradation of circulating von Willebrand factor and its regulator ADAMTS13 implicates secreted Bacillus anthracis metalloproteases in anthrax consumptive coagulopathy. J Biol Chem 2008-04-01 [PMID: 18263586]

Tao Z, Peng Y, Nolasco L et al. Recombinant CUB-1 domain polypeptide inhibits the cleavage of ULVWF strings by ADAMTS13 under flow conditions. Blood 2005-12-01 [PMID: 16141351]

Dong JF. Cleavage of ultra-large von Willebrand factor by ADAMTS-13 under flow conditions. J Thromb Haemost 2005-08-01 [PMID: 16102037]

Tao Z, Wang Y, Choi H et al. Cleavage of ultralarge multimers of von Willebrand factor by C-terminal-truncated mutants of ADAMTS-13 under flow. Blood 2005-07-01 [PMID: 15774619]



Procedures

Serum protocol for ADAMTS13 Antibody (NB100-584)

Nuclear Extract and Cytoplasmic Fraction Preparation protocol for ADAMTS13 Antibody (NB100-584):

Nuclear Extract and Cytoplasmic Fraction Preparation:

1. Nuclear extracts (NE) and cytoplasmic fractions (S100) were prepared by Dignam's method (Dignam, Lebovitz, and Roeder, *Nucleic Acids Res.* 11: 1475-1489. 1983).
2. 100 liters of HeLa cell culture were harvested and washed 3 times with cold PBS.
3. The packed-cell volume (PCV) was measured, and the cell pellet was gently resuspended with 5 PCVs of hypotonic buffer (10 mM HEPES-KOH [pH 8], 10 mM KCl, 1.5 mM MgCl₂, 1 mM DTT, 0.2 mM PMSF).
4. Cells were incubated on ice for 10 minutes and then pelleted by centrifugation at 1,800xg for 10 minutes.
5. Hypotonic buffer was added to 2 PCVs, and cells were resuspended and then homogenized with 15 strokes using a pestle B in a Dounce glass homogenizer until the cells were more than 90% lysed, as determined by a light microscope.
6. The lysate was centrifuged at 20,000xg for 30 minutes at 4 degrees Celcius.
7. The supernatant was saved for S100 fraction, and the pellet was saved to measure the packed nuclear volume (PNV).
8. 0.4 ml of extraction buffer (20 mM HEPES-KOH [pH 8], 0.6 M KCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 25% [vol/vol] glycerol, 1 mM DTT, 0.2 mM PMSF) per ml of PNV was added.
9. Cell nuclei were homogenized with 10 strokes of pestle A in the homogenizer.
10. Suspension was stirred at 4 degrees Celcius for 30 minutes and centrifuged for 30 minutes at 20,000xg.
11. The supernatant (nuclear extract) was aliquotted for use.
12. The S100 fraction (resulting supernatant) was mixed with 0.11 volume of high-salt buffer (20 mM HEPES-KOH [pH 8], 1.2 M KCl, 1.5 mM MgCl₂, 0.2 mM EDTA, 20% [vol/vol] glycerol, 1 mM DTT, 0.2 mM PMSF) and centrifuged at 100,000xg for 60 minutes at 4 degrees Celcius.
13. This supernatant was dialyzed for 2 hours at 4 degrees Celcius.
14. The sample was centrifuged for 30 minutes at 20,000xg and the supernatant (S100) was aliquotted for use.

Immunoprecipitation Antibody Characterization:

1. HeLa NE and S100 were diluted with 1 volume of RIPA buffer [150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris [pH 8]].
2. Cleared by spinning at 100,000 g for 20 minutes at 4 degrees Celcius.
3. 1 ml of supernatant (~10 mg total protein) was mixed with 20 ug of primary antibody (NB 100-584) and rotated overnight at 4 degrees Celcius.
4. Supernatant was mixed with 0.05 ml of protein A-sepharose beads (50% slurry) and rotated for 2 hours at 4 degrees Celcius.
5. Immunoprecipitates were washed 3 times with the 10% RIPA in PBS.
6. The washed beads were boiled with 0.04 ml of Laemmli buffer and subjected to SDS-PAGE (4-20% Tris-glycine gel).

Complex purification:

1. NE and S100 were cleared by spinning at 20,000 g for 30 minutes at 4 degrees Celcius.
2. 1.5 ml of supernatant (~15 mg total protein) was mixed with 20 ug of primary antibody (NB 100-584) and rotated for 4 hours at 4 degrees Celcius.
3. Sample and antibody mixture were centrifuged at 15,000 g for 20 minutes at 4 degrees Celcius.
4. Supernatant was mixed with 0.05 ml of protein A-sepharose beads (50% slurry) and rotated for 1 hour at 4 degrees Celcius.
5. Immunoprecipitates were washed 3 times with the NETN buffer (20 mM Tris-HCl [pH 8], 100 mM NaCl, 1 mM EDTA, 0.5% NP-40).
6. The washed beads were boiled with 0.04 ml of Laemmli buffer and subjected to SDS-PAGE (4-20% Tris-glycine gel).

*If an insufficient amount of protein is purified for identification from 15 mg of extract, carry out the same procedure using 50-100 mg of extract to increase the amount of purified protein yield.



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Products Related to NB100-584

HAF017	Rabbit anti-Goat IgG Secondary Antibody [HRP (Horseradish Peroxidase)]
HAF109	Donkey anti-Goat IgG Secondary Antibody [HRP (Horseradish Peroxidase)]
NB410-28088-1mg	Goat IgG Isotype Control
NBP2-14266PEP	ADAMTS13 Recombinant Protein Antigen

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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