Product Datasheet

TEM8/ANTXR1 Antibody (200C1339(SB20)) - BSA Free NB100-56585SS

Unit Size: 0.025 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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Publications: 5

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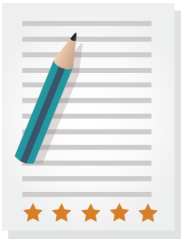


NB100-56585SS

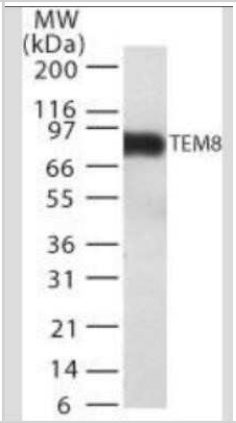
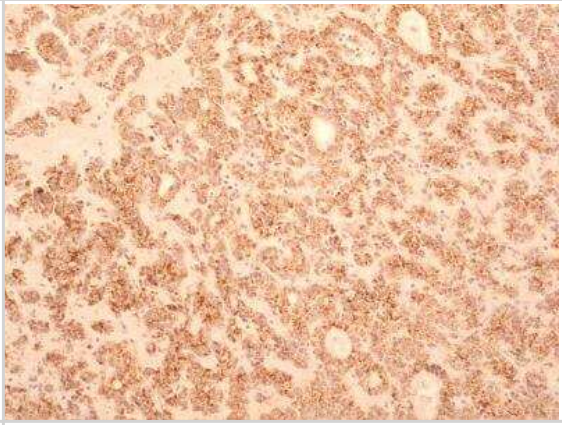
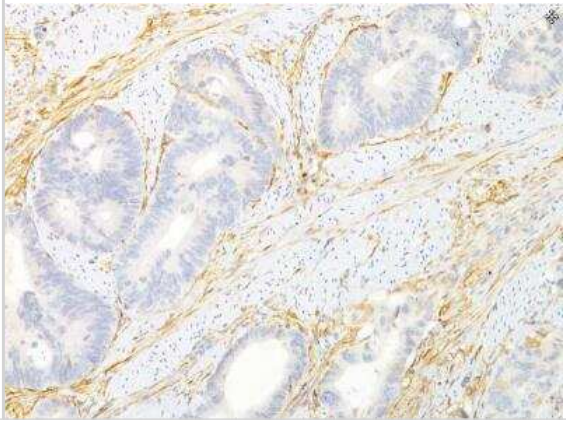
TEM8/ANTXR1 Antibody (200C1339(SB20)) - BSA Free

Product Information	
Unit Size	0.025 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	200C1339(SB20)
Preservative	0.02% Sodium Azide
Isotype	IgG1
Purity	Protein G purified
Buffer	PBS

Product Description	
Host	Mouse
Gene ID	84168
Gene Symbol	ANTXR1
Species	Human
Specificity/Sensitivity	Nanda et al (2004) note a passage dependent loss of TEM8 in HUVEC suggesting that that TEM8 may be a specific marker of the endothelial cell (EC) phenotype which is lost as primary EC's age in culture and de-differentiate.
Immunogen	This monoclonal antibody was developed by immunizing mice with a synthetic peptide containing amino acids 481-497 (CINFTRVKNNQPAKYPL) of human TEM8.

Product Application Details	
Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1-3 ug/ml, Immunohistochemistry 2-5 ug/ml, Immunocytochemistry/ Immunofluorescence reported in scientific literature (Matsuda (2010)), Immunohistochemistry-Paraffin 2-5 ug/ml. Use reported in scientific literature (Hotchkiss et al (2005))
Application Notes	TEM8 is observed at ~80 kDa in western blots of HUVEC cells (Hotchkiss et al, 2005) and as an 80 kDa and 85 kDa double in human colon cell lysates (Nanada et al, 2004). The size difference is thought to be due to glycosylation as a 70 kDa band was seen when cell extracts in Nanda et al (2004) were treated with a glycosidase cocktail.

Images

Western Blot: TEM8/ANTXR1 Antibody (200C1339(SB20)) [NB100-56585] - Detection in TEM8 transfected cell lysate using this antibody.	
Immunohistochemistry-Paraffin: TEM8/ANTXR1 Antibody (200C1339 (SB20)) [NB100-56585] - Detection of TEM8/ANTXR1 in human liver cancer section using 5 ug/ml concentration of TEM8 antibody. Uniform weak staining was observed in the cancerous cells, while the blood vessels depicted the expected intense positivity for TEM8.	
Western Blot: TEM8/ANTXR1 Antibody (200C1339(SB20)) [NB100-56585] - Analysis of human heart lysates (35ug per lane, RIPA buffer) using TEM8 antibody (NB100-56585) at 0.3ug/ml. Band observed at 65kDa. (Expected MW of 62.8kDa according to NP_115584.1).	
Immunohistochemistry-Paraffin: TEM8/ANTXR1 Antibody (200C1339 (SB20)) [NB100-56585] - Human colon stained with TEM8 antibody at 2.5 ug/ml concentration using DAB chromogen detection method (10X magnification)	

Publications

Kieffer Y, Hocine HR, Gentric G et al. Single-cell analysis reveals fibroblast clusters linked to immunotherapy resistance in cancer Cancer Discov 2020-05-20 [PMID: 32434947] (FLOW, Human)

Nanda A, Carson-Walter EB, Seaman S et al. TEM8 interacts with the cleaved C5 domain of collagen alpha 3(VI). Cancer Res. 2004-02-01 [PMID: 14871805] (WB, Human)

Details:
WB: TEM8 transfected/non-transfected 293 cells (Fig 1A and B), human colon cancer tissue lysates and patient-matched normal colonic mucosa transfected (Fig 1C). Note: antibody specificity is validated by using TEM8 transfected/non-transfected 293 cells in

Hotchkiss KA, Basile CM, Spring SC et al. TEM8 expression stimulates endothelial cell adhesion and migration by regulating cell-matrix interactions on collagen. Exp Cell Res. 2005-04-15 [PMID: 15777794] (WB, IHC-P, Human)

Details:
IHC (Paraffin): Fig 1A (human umbilical cords); WB: Fig 1C and 1D [human venous epithelial cells (HUVEC)].

Matsuda K, Ohga N, Hida Y et al. Isolated tumor endothelial cells maintain specific character during long-term culture. Biochem Biophys Res Commun. 2010-04-16 [PMID: 20302845] (ICC/IF, Human)

Details:
IF/ICC: primary human xenograft tumor endothelial cell cultures (Fig 2B).

Werner E, Kowalczyk AP, Faundez V. Anthrax toxin receptor 1/tumor endothelium marker 8 mediates cell spreading by coupling extracellular ligands to the actin cytoskeleton. J Biol Chem. 2006-08-11 [PMID: 16762926] (WB)

Details:
WB: TEM8 transfected/non-transfected 293 cells (Figs 1A, 4A, and 5D). Note: antibody specificity is validated by using TEM8 transfected/non-transfected 293 cells in Figs 1A and 4A.

Procedures

Western Blot Protocol for TEM8/ANTXR1 Antibody (NB100-56585)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturer's instructio

Immunohistochemistry-Paraffin Protocol for TEM8/ANTXR1 Antibody (NB100-56585)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer at all times).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

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