Datasheet for ABIN1866534

anti-ADAM8 antibody (AA 145-493)

1 Validation

3 Images

Go to Product page

Overview

Quantity:	100 µL
Target:	ADAM8
Binding Specificity:	AA 145-493
Reactivity:	Mouse
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This ADAM8 antibody is un-conjugated
Application:	Western Blotting (WB), Immunohistochemistry (IHC), Immunoprecipitation (IP), Immunocytochemistry (ICC)

Product Details

Purpose:	Polyclonal Antibody to A Disintegrin And Metalloprotease 8 (ADAM8)
Immunogen:	Recombinant A Disintegrin And Metalloprotease 8 (ADAM8) corresdonding to Glu145~Cys493 with N-terminal His Tag
Isotype:	IgG
Specificity:	The antibody is a rabbit polyclonal antibody raised against ADAM8. It has been selected for its ability to recognize ADAM8 in immunohistochemical staining and western blotting.
Cross-Reactivity:	Human, Rat
Purification:	Antigen-specific affinity chromatography followed by Protein A affinity chromatography

Target Details

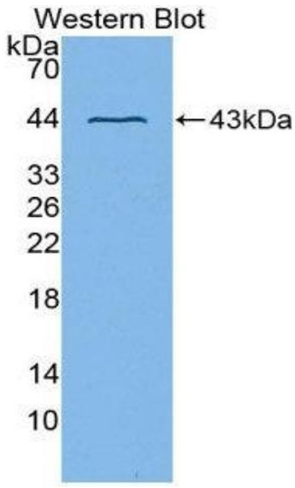
Target:	ADAM8
Alternative Name:	A Disintegrin And Metalloprotease 8 (ADAM8 Products)
Background:	CD156-A, MS2, CD156a, Disintegrin and metalloproteinase domain-containing protein 8
Pathways:	Activation of Innate immune Response, M Phase

Application Details

Application Notes:	Western blotting: 0.5-5 µg/mL Immunohistochemistry: 5-20 µg/mL Immunocytochemistry: 5-20 µg/mL Optimal working dilutions must be determined by end user.
Comment:	The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.
Restrictions:	For Research Use only

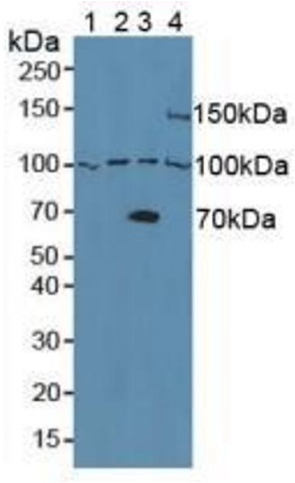
Handling

Format:	Liquid
Concentration:	Lot specific
Buffer:	0.01M PBS, pH 7.4, containing 0.05 % Proclin-300, 50 % glycerol.
Preservative:	ProClin
Precaution of Use:	WARNING: Reagents contain sodium azide. Sodium azide is very toxic if ingested or inhaled. Avoid contact with skin, eyes, or clothing. Wear eye or face protection when handling. If skin or eye contact occurs, wash with copious amounts of water. If ingested or inhaled, contact a physician immediately. Sodium azide yields toxic hydrazoic acid under acidic conditions. Dilute azide-containing compounds in running water before discarding to avoid accumulation of potentially explosive deposits in lead or copper plumbing.
Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	4 °C,-20 °C
Storage Comment:	Store at 4°C for frequent use. Stored at -20°C in a manual defrost freezer for two year without detectable loss of activity. Avoid repeated freeze-thaw cycles.
Expiry Date:	24 months



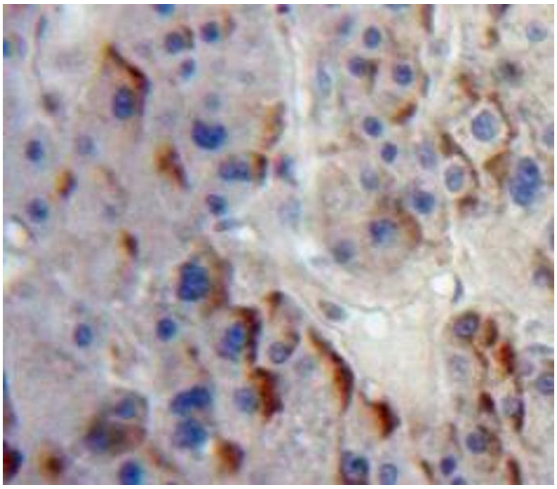
Western Blotting

Image 1.



Western Blotting

Image 2. Figure. Western Blot; Sample: Lane1: Human Raji Cells; Lane2: Human Raw264.7 Cells; Lane3: Human 293T Cells; Lane4: Mouse Pancreas Tissue.



Immunohistochemistry

Image 3. Used in DAB staining on fromalin fixed paraffin-embedded Liver tissue

Validation report #104303 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by Department of Neurosurgery, Philipps-University Marburg

Report Number: 104303

Date: Feb 11 2021

Target:	ADAM8
Lot Number:	PAA620Mu01
Method validated:	Western Blotting (WB)
Positive Control:	Wildtype bone marrow derived mouse macrophages
Negative Control:	Adam8 knock-out bone marrow derived mouse macrophages
Notes:	Passed. ABIN1866534 specifically recognizes Adam8 in lysates of bone marrow derived mouse macrophages.
Primary Antibody:	ABIN1866534
Secondary Antibody:	ABIN269949
Protocol:	• Isolate and subsequently grow Adam8 wt or ko bone marrow derived mouse macrophages in DMEM (Gibco, 11965-092, lot 2258599) supplemented with FBS superior (Sigma Aldrich, S0615, lot 0001640840), Pen Strep (Gibco, 15140-122, lot 2240835), and Murine M-CSF (PeproTech, 315-02, lot 0419245) at 37°C and 5% CO_2 in 2 mL on a 6 well plate to 70% confluency. • Lyse 800,000 cells in 50 µL RIPA buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 1% (v/v) NP-40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS, 10 mM phenanthrolin, 10 mM EDTA, phosphatase and proteinase inhibitor). • Determine total protein content of the lysates using Pierce BCA Protein Assay Kit (Thermo Scientific, 23225, lot RH237553). • Denature 10 µg of total protein for 5 min at 95 °C in 1x Laemmli Buffer and subsequently separate them on a denaturing 10% SDS PAGE gel (polyacrylamide, Tris-HCl pH 6.8 for the stacking gel and pH 8.8 for separating gel, water, ammonium persulfate, SDS, tetramethyldiamine in a Mini-PROTEAN Tetra System (BioRad) for 1.5 h at 120 V. • Transfer proteins onto nitrocellulose membrane (GE Healthcare Life Science, 10600001, lot A29635973) with a Western blotting system for 70 min at 150 mA. • Block the membrane with TBST containing 5% milk or 5% BSA for 1 h at RT. • Incubation with primary ◦ rabbit anti-ADAM8 antibody (antibodies-online, ABIN1866534, PAA620Mu01) diluted 1:1,000 in TBST containing 5% milk or 5% BSA for over night at 4°C. ◦ rabbit-anti TUBB antibody (Novus Biologicals, NB600-936, lot J) diluted 1:2,000 in TBST

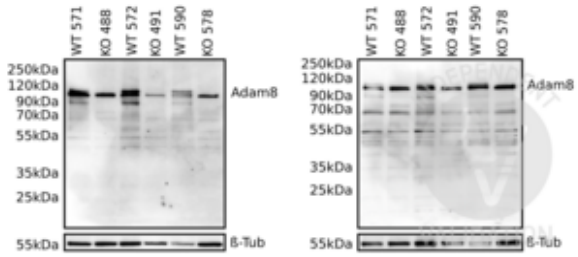
containing 5% milk over night at 4°C.

- Wash membrane 3x for 10 min with TBST.
- Incubation with secondary Donkey Anti-Rabbit IgG H&L (HRP) (abcam, ab97064, lot GR3209122-1) diluted 1:5,000 in TBST containing 5% milk for 1h at RT.
- Wash membrane 3x for 10 min with TBST.
- Reveal protein bands using Western Bright Chemilumineszenz Substrat Sirius (Biozym, 541021, no 200331-75) on a Developing machine, Royal Intas ChemoCam Imager (Inta Science Imaging) exposure time 20 sec (TBST with 5% milk) or 3 sec (TBST with 5% BSA).

Experimental Notes:

- ABIN1866534 reveals protein bands and 90 kDa and 110 kDa specifically in the Adam8 wildtype lysates. They are not present in the Adam8 ko lysates. And additional unspecific band between these two bands in visible in all lysates.
- Blocking with TBST containing 5% milk gives clearer results that blocking with TBST containing 5% BSA.

Image for Validation report #104303



Validation image no. 1 for anti-ADAM Metallopeptidase Domain 8 (ADAM8) (AA 145-493) antibody (ABIN1866534)

Western blot analysis of cell lysates from wildtype (WT) or Adam8 knock-out (KO) bone marrow derived mouse macrophages with ABIN1866534 (upper panels) or a beta-tubulin loading control (lower panels). Primary antibody incubation in TBST containing 5% milk (left) or 5% BSA (right).