Datasheet for ABIN129524

anti-JAG1 antibody (Internal Region)

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Overview

Quantity:	500 µg
Target:	JAG1
Binding Specificity:	Internal Region
Reactivity:	Human, Mouse
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This JAG1 antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunohistochemistry (IHC), Fluorescence Microscopy (FM)

Product Details

Immunogen:	This protein A purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to amino acids 110-125 of human Jagged-1protein. Immunogen Type: Peptide
Isotype:	IgG
Specificity:	This protein A purified antibody is directed against human Jagged-1 protein. The product was purified from mono-specific antiserum by affinity chromatography. A BLAST analysis was used to suggest cross reactivity with Jagged-1 protein from human, chimpanzee, rat and mouse based on 100% homology with the immunizing sequence. Partial reactivity is expected against dog (81%) and Xenopus laevis (85%) based on partial sequence homologies as indicated. Reactivity against homologues from other sources is not known.

Product Details

Characteristics:	Anti Jagged 1 Antibody recognizes the jagged 1 protein encoded by the JAG1 gene, that is the human homolog of the Drosophilia jagged protein. Human jagged 1 is the ligand for the receptor notch 1, the latter a human homolog of the Drosophilia jagged receptor notch. Mutations that alter the jagged 1 protein cause Alagille syndrome. Jagged 1 signaling through notch 1 has also been shown to play a role in hematopoies.
Purification:	purified
Sterility:	Sterile filtered

Target Details

Target:	JAG1
Alternative Name:	Jagged 1 (JAG1 Products)
Background:	Anti Jagged 1 Antibody recognizes the jagged 1 protein encoded by the JAG1 gene, that is the human homolog of the Drosophilia jagged protein. Human jagged 1 is the ligand for the receptor notch 1, the latter a human homolog of the Drosophilia jagged receptor notch. Mutations that alter the jagged 1 protein cause Alagille syndrome. Jagged 1 signaling through notch 1 has also been shown to play a role in hematopoies. Synonyms: Ser 1 antibody, AGS antibody, AHD antibody, AWS antibody, CD 339 antibody, CD339 antibody, CD339 antigen antibody, Headturner antibody, HJ1 antibody, Htu antibody
Gene ID:	182, 4557679
UniProt:	P78504
Pathways:	Notch Signaling, Stem Cell Maintenance

Application Details

Application Notes:	This protein A purified antibody has been tested for use in ELISA, immunohistochemistry, immunofluorescence microscopy and western blot. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 150 kDa in size corresponding to Jagged-1 in mouse liver whole cell lysates, and a 75 kDa band in human brain and kidney lysates.
Comment:	Gene Name: JAG1
Restrictions:	For Research Use only

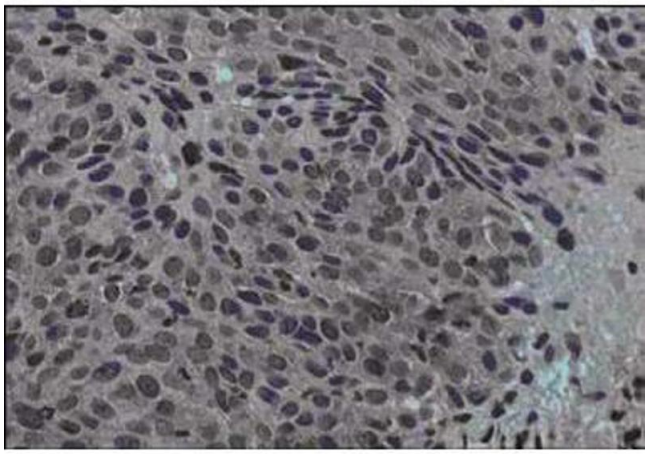
Handling

Format:	Liquid
Concentration:	1.1 mg/mL
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	Sodium azide
Precaution of Use:	This product contains sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C/-20 °C
Storage Comment:	Store vial at 4 °C prior to restoration. For extended storage aliquot contents and freeze at -20 °C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4 °C as an undiluted liquid. Dilute only prior to immediate use. Expiration date is one (1) year from date of opening.
Expiry Date:	12 months

Publications

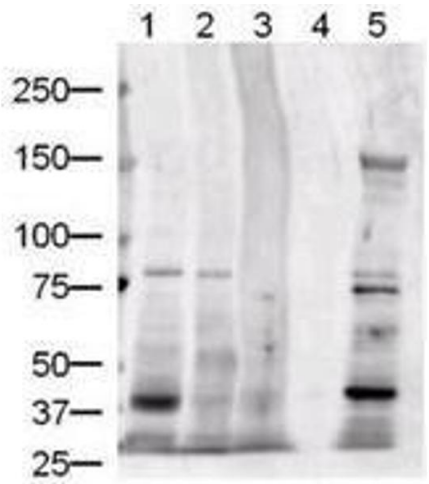
Product cited in:	Satoh, Nakano, Shibata, Maki: "The penta-EF-hand domain of ALG-2 interacts with amino-terminal domains of both annexin VII and annexin XI in a Ca2+-dependent manner." in: **Biochimica et biophysica acta**, Vol. 1600, Issue 1-2, pp. 61-7, (2002) (PubMed).

Images



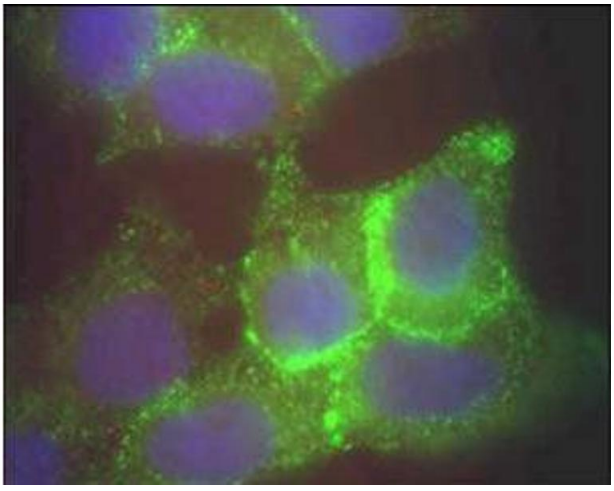
Immunohistochemistry

Image 1. Immunohistochemical staining of human cervical cancer tissue (40X magnification) using Protein A purified anti-Jagged-1 antibody. Tissue was fixed with formalin and embedded in paraffin. Hematoxylin was used to counter-stain cells. A 1:100 dilution of primary antibody was used. Personal Communication. Martin Kast Laboratory.



Western Blotting

Image 2. Western blot using Protein A purified anti-Jagged-1 antibody shows detection of Jagged-1 protein in various whole cell lysates: human brain (lane 1), human kidney (lane 2), human liver (lane 3), and mouse liver (lane 5). Lane 4 contained sample buffer only. The band at ~134 kDa in lane 5 is believed to be Jagged-1 precursor. The identity of minor reactive bands is unknown. Each lane contains approximately 20 ug of lysate. Primary antibody was used at a 1:500 dilution. The membrane was washed and reacted with a 1:5,000 dilution of HRP conjugated Gt-a-Rabbit IgG. Exposure time was 1 min. Predicted molecular weight is 134 kDa.



Immunofluorescence

Image 3. Immunofluorescence microscopy using Protein A purified anti-Jagged-1 antibody of human corneal epithelial cells. Primary antibody was used at a 1:500 dilution. The Jagged1 (green staining) is local-ized to the cytoplasm and is consistent with reports in the literature. The nucleus is stained with Bis benzimide (blue). Personal Communication. Aihua Ma, Univdersity of Cardiff.

Validation report #103702 for Western Blotting (WB)

Successfully validated (Western Blotting (WB))

by AG Pancreatic Development and Stem cell differentiation, Universitätsklinikum Ulm

Report Number: 103702

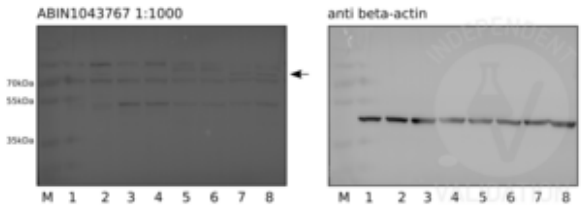
Date: Jun 16 2019

Target:	JAG1
Lot Number:	28285
Method validated:	Western Blotting (WB)
Positive Control:	human pluripotent stem cells (day 0) pancreatic progenitor cells (day 14)
Negative Control:	definite endoderm cells (day 4) pancreatic endoderm cells (day 10)
Notes:	ABIN1043767 reveals a protein band of the expected MW and some weaker extraneous bands in cell lysates of human pluripotent stem cells and pancreatic progenitor cells.
Primary Antibody:	ABIN1043767
Secondary Antibody:	donkey anti-rabbit HRP-conjugated antibody (GE Healthcare, NA9310V)
Protocol:	• Grow HUES8 in mTeSR1 (STEMCELL Technologies, 85850) at 37°C and 5% O_2, 5% CO_2 in 2ml in a 6-well plate to 90% confluency. • Harvest cells using TrypLE (ThermoFisher Scientific, 12604013) following the manufacturer´s instructions. • Lyse 2x10^6 cells in 30µl per well cold RIPA buffer (50mM Tris-HCl pH 8.0, 150mM NaCl, 0.1% SDS, 0.5% deoxycholate, 1% TritonX 100 in ddH_2O) supplemented with 1mM PMSF and 1x protease inhibitor (Roche, 11836170001) for 30min on ice with 3x vortexing in 1.5ml microcentrifuge tubes. • Centrifuge tubes at 10000xg for 8min at 4°C. • Transfer supernatant to a new 1.5ml microcentrifuge tube and store at -80°C. • Determine total protein content of the lysates using a Bradford assay (Bio-Rad, 500-0006). • Denature 50µg of total protein for 5min at 95°C in 30µl 1x Laemmli SDS sample buffer and subsequently separate them on a denaturing 7.5% polyacrylamide gel (7.5% Acrylamide, 0.375M Trís-HCl (pH8.8), 0.1% SDS, 0.1% APS, 0.1% TEMED) for about 90min at 120V. • Transfer proteins onto a PVDF membrane (Sigma Aldrich, IPVH00010) with transfer buffer (5.27g Tris, 2.93 g glycerine, 200 ml methanol, fill to 1l ddH_2O) in a semidry western blotting system for 75min at 80mA/gel. • Check transfer of the separated proteins by Ponceau S staining.

- Rinse membrane with water.
- Wash membrane for 5min with TBST (TBS, 0.02% Tween20).
- Block the membrane with 20ml blocking buffer (TBST, 5% milk) on a shaker for 1h at RT.
- Rinse membrane 3x with TBST.
- Wash membrane on a shaker 3x for 5min with TBST.
- Shrink-wrap and incubate membrane with primary
  - rabbit anti-JAG1 antibody (antibodies-online, ABIN1043767, lot 28285) diluted 1:1000 and 1:10000 respectively in blocking buffer ON at 4°C or
  - mouse anti-beta actin antibody (Sigma Aldrich, A1978) diluted 1:2000 in blocking buffer at RT for 1h.
- Wash membrane 3x for 5min with TBST.
- Incubate membrane with secondary
  - donkey anti-rabbit HRP-conjugated antibody (GE Healthcare, NA9310V) diluted 1:5000 in TBST containing 1% milk for 1h at RT or
  - donkey anti-mouse HRP-conjugated antibody (GE Healthcare, NA9340V) diluted 1:5000 in TBST containing 1% milk for 1h at RT.
- Wash membrane 3x for 5min with TBST.
- Reveal protein bands using ECL solution (ThermoScientific, 34076) on a Fusion SL (Vilber) chemiluminescence system. Exposure times for the 1:1000 and 1:10000 dilutions of ABIN1043767 were 30sec and 5min respectively.

Experimental Notes:

- The bands marked by the arrow in the western blot image using ABIN1043767 at a 1:1000 dilution seem to be specific, as JAG1 showed highest transcriptomic levels at pancreatic progenitor stage (lanes 7 and 8), followed by pluripotent stem cell state (lane 2). Although the immunoblot with ABIN1043767 results in some unspecific bands, the expected band height fits nicely (75kDa). Higher MW bands might potentially represent precursor proteins detectable in all samples but pancreatic progenitor stage samples. In principle specific bands are also detectable with ABIN1043767 at a 1:10000 dilution, although exposition time was very long.
- ABIN1043767 was also tested in IHC on 4-5µm FFPE sections of primary human kidney cancer tissue. Epitope retrieval was carried out using Tris-EDTA buffer at pH9.0 (Zytomed, ZUC029-500), EDTA at pH8.0 (Leica, RE7116), or citrate buffer at pH6.1 (Agilent, S169984-2) for 20min in a decloaking chamber. Antigen retrieval at pH8.0 looks ok, assuming a broad expression pattern. However, detection would be rather expected in fewer tubular cells. No staining was observed in glomeruli. Cytoplasmic staining in tubuli is revealed at higher magnification. Note that antigen retrieval influences the staining pattern. All in all, staining needs to be validated for example in protein ablation assays.



Validation image no. 1 for anti-Jagged 1 (JAG1) (Internal Region) antibody (ABIN1043767)

Western blot with ABIN1043686 on human pluripotent stem cells (1 and 2), definite endoderm cells (3 and 4), pancreatic endoderm cells (5 and 6), and pancreatic progenitors (7 and 8). ABIN1043686 was used at a 1:1000 (30sec exposure) or 1:10000 dilution (5min exposure). Beta-actin served as loading control.