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Datasheet for 200-401-A57

TMBIM1 Antibody

Overview

Description:	Anti-TMBIM1 (RABBIT) Antibody - 200-401-A57
Item No.:	200-401-A57
Size:	100 µg
Applications:	ELISA, IHC, WB
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. TMBIM1 (Transmembrane BAX inhibitor motif-containing protein 1) is a member of the 7 TMS (7 transmembrane domains) family of receptors known to mediate the activation of various transcription factors. TMBIM1 is identified as a novel modulator of NF-kB activation. A drosophila homolog of this protein (dNMDA1 with 40% homology to hTMBIM1) is reported to be unregulated during aging and oxidative stress.
Synonyms:	rabbit anti-TMBIM1 Antibody, Putative uncharacterized protein TMBIM1, Protein lifeguard 3, Protein RECS1 homolog, Transmembrane BAX inhibitor motif-containing protein 1, LFG3, RECS1, PP1201, PSEC0158
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	TMBIM1
Reactivity:	Human
Immunogen Type:	Recombinant Protein

Immunogen:	This protein A purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a recombinant protein corresponding to the amino terminus of human TMBIM1 protein.
Purity/Specificity:	This product was protein A purified from monospecific antiserum by immunoaffinity chromatography using protein A coupled to agarose beads. This antibody is specific for human TMBIM1 protein. A BLAST analysis was used to suggest partial cross-reactivity with TMBIM1 from mouse and rat sources based on ~88% homology with the immunizing sequence. Cross-reactivity with TMBIM1 from other sources has not been determined.
Relevant Links:	• NCBI - 50593008 • UniProtKB - Q969X1 • GeneID - 64114

Application Details

Tested Applications:	ELISA, IHC, WB
Application Note:	This protein A purified antibody has been tested for use in ELISA, Immunohistochemistry, and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately 35 kDa in size corresponding to TMBIM-1 by western blotting in the appropriate cell lysate or extract. To date this antibody has shown the ability to recognize over-expressed TMBIM1 but not endogenous protein.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000
IHC:	10µg/mL
WB:	1:500 - 1:2,000

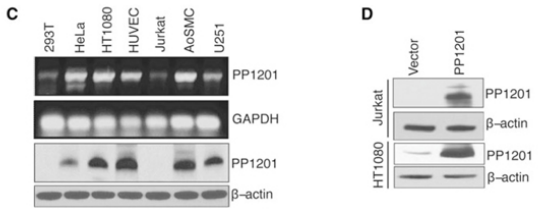
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.2 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

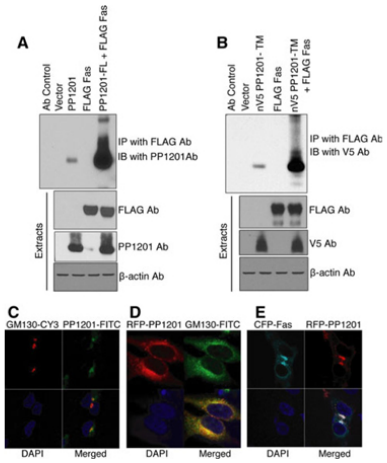
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



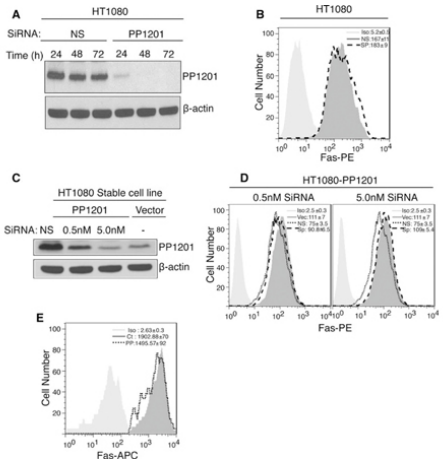
Western Blot

c (TMBIM1) PP1201 is expressed by various cell lines. Semi-quantitative RT-PCR was performed to quantify the expression level of PP1201 in different cell lines (Upper Panel). Amplifications of glyceraldehyde-3-phosphate dehydrogenase were used as loading controls. Lower panels show endogenous protein levels in corresponding cell lines and β-Actin was used as loading control for protein. 30 µg of protein was loaded in each well. d Generation of stable cell lines. Using retroviral transfection (As described in "Materials and methods") Jurkat and HT1080 cell lines were used to stably express PP1201. Endogenous expression of PP1201 was present in HT1080 and absent in Jurkat. β-Actin was used as loading control for protein. Fig 1.
PMID: 21107705



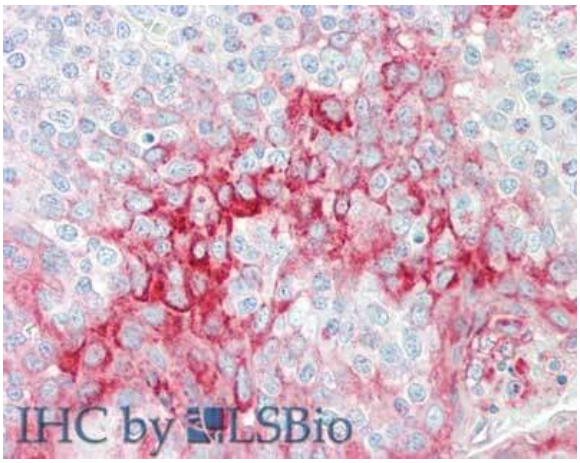
Western Blot

PP1201 interacts with Fas mainly through transmembrane region and localizes into intracellular compartment. a Coimmunoprecipitation of PP1201 with FLAG Fas. Expression plasmids for PP1201 and FLAG Fas were transfected in combination and alone in 293 cells. Cell lysates were immunoprecipitated with mouse anti-FLAG and immunoblotted with rabbit anti-PP1201. Lysates were immunoblotted with anti-FLAG, anti-PP1201 and anti β-actin. b Coimmunoprecipitation of N-terminal tagged V5 truncated PP1201-TM (expressing transmembrane region, 103–311aa) and FLAG Fas. Expression plasmids of these two were transfected in 293-T cell in combination and alone. Cell lysates were immunoprecipitated with mouse anti-FLAG and immunoblotted with mouse anti V5. Lysates were immunoblotted with anti-FLAG, anti-V5 and anti β-actin. c Immunostaining for PP1201 at endogenous level in HUVEC cells using GM130 as a golgi marker shows that PP1201 partially localized into golgi. d Transient transfection of RFP tagged PP1201 in Hela cells and staining with golgi marker GM130 shows PP1201 to be localized into golgi compartment. e Transient transfection of CFP (Cyan Fluorescent Protein) tagged Fas along with RFP tagged PP1201 shows colocalization of both mostly into perinuclear spaces like Golgi. Fig 3.
PMID: 21107705

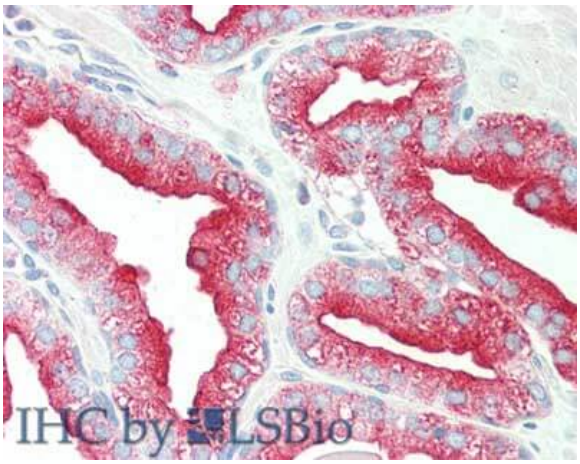


Western Blot

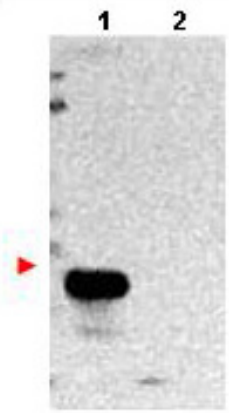
Silencing of endogenous PP1201 does not influence Cell surface Fas expression but overexpressed PP1201 does. a Endogenous expression of PP1201 goes down up to 72 h upon silencing in HT1080 cells. 50 nM of ON-TARGET plus SMART pool (Thermo scientific) specific to human PP1201 was transfected using Lipofectamine RNAiMAX (Invitrogen). As a control 50 nM Non-targeting pool was used. After transfections lysates were prepared of transfected cells for transfection period of 24 h, 48 h and at 72 h and subjected to immunoblot with anti-PP1201 antibody. Blotting with β-actin shows loading control. 30 µg of protein was loaded in each lane. b Flow cytometry analysis shows cell surface level of Fas in HT1080 after silencing of endogenous PP1201 at 48 h. Overlay histogram represents one of the triplicate and inset shows average of mean fluorescence intensities (±SD) of triplicate values for Fas staining. c PP1210 expressing HT1080 cells were transfected with the indicated siRNAs and 30 µg of cellular lysates was probed with the indicated proteins. d Flow cytometry analysis exhibits surface levels of Fas after silencing to various levels of overexpressed PP1201 in HT1080 stable cell line. Overlay histogram represents one of the triplicates and inset shows average of mean fluorescence intensities (±SD) of triplicate values for Fas staining. e Overexpression of PP1201 in AoSMC cells down regulates the cell surface expression of Fas. AoSMC cells were transfected with 1 µg eGFP either with 5 µg vector (control) or with 5 µg PP1201 expression plasmid and after 48 h cells were stained with Fas antibody conjugated with APC and double positive cells were analyzed by FACS. Double positive populations were selected and Fas staining was analyzed by overlay histogram plot and by comparing mean fluorescence intensities. Overlay histogram represents one of the triplicates and inset shows average of mean fluorescence intensities (±SD) of triplicate values for Fas staining. Fig 5. PMID: 21107705



Immunohistochemistry
Immunohistochemistry of Rabbit anti-TMBIM1 antibody. Tissue: human tonsil. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: anti-TMBIM1 antibody at 10 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Staining: TMBIM1 as precipitated red signal with hematoxylin purple nuclear counterstain.



Immunohistochemistry
Immunohistochemistry of Rabbit anti-TMBIM1 antibody. Tissue: human prostate. Fixation: formalin fixed paraffin embedded. Antigen retrieval: not required. Primary antibody: anti-TMBIM1 antibody at 10 µg/mL for 1 h at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:10,000 for 45 min at RT. Staining: TMBIM1 as precipitated red signal with hematoxylin purple nuclear counterstain.



Western Blot
Western blot using Rockland's protein A purified anti-TMBIM1 antibody shows detection of exogenous TMBIM1 in lysates from HeLa cells transfected with pcDNA3-hTMBIM1 (lane 1). No staining is observed in lysates from mock transformed HeLa cells (lane 2). To date this antibody has shown the ability to recognize over expressed TMBIM1 ~35kDa, but not endogenous protein. The membrane was probed with the primary antibody at a 1:1,000 dilution at 4° C, overnight. Personal Communication from Srinivasa Srinivasula, CCR-NCI, Bethesda, MD.

References

- Shukla, S et al. A shear stress responsive gene product PP1201 protects against Fas-mediated apoptosis by reducing Fas expression on the cell surface. *Apoptosis : An International Journal on Programmed Cell Death* (2011)

Disclaimer

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