Datasheet for ABIN405704

anti-RHOT1 antibody (N-Term)

1 Validation

1 Image

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Overview

Quantity:	100 µg
Target:	RHOT1
Binding Specificity:	N-Term
Reactivity:	Human, Mouse, Rat, Dog, Rabbit, Cow, Guinea Pig, Horse, Zebrafish (Danio rerio)
Host:	Rabbit
Clonality:	Polyclonal
Application:	Western Blotting (WB)

Product Details

Immunogen:	The immunogen is a synthetic peptide directed towards the N terminal region of human RHOT1
Sequence:	EMKPACIKAL TRIFKISDQD NDGTLNDAEL NFFQRICFNT PLAPQALEDV
Predicted Reactivity:	Cow: 100%, Dog: 100%, Guinea Pig: 100%, Horse: 100%, Human: 100%, Mouse: 100%, Rabbit: 100%, Rat: 100%, Zebrafish: 85%
Characteristics:	This is a rabbit polyclonal antibody against RHOT1. It was validated on Western Blot using a cell lysate as a positive control.
Purification:	Affinity Purified

Target Details

Target:	RHOT1
Alternative Name:	RHOT1 (RHOT1 Products)

Target Details

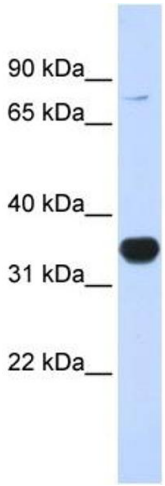
Background:	RHOT1 is mitochondrial GTPase involved in mitochondrial trafficking. It is probably involved in control of anterograde transport of mitochondria and their subcellular distribution. Alias Symbols: ARHT1, FLJ11040, FLJ12633, MIRO-1, MIRO1 Protein Interaction Partner: PCSK9, ERLIN2, PARK2, ILK, UBC, CFTR, PINK1, UBD, IRAK1, AMFR, Protein Size: 659
Molecular Weight:	75 kDa
Gene ID:	55288
NCBI Accession:	NM_001033566, NP_001028738
UniProt:	Q8IXI2

Application Details

Application Notes:	Optimal working dilutions should be determined experimentally by the investigator.
Comment:	Antigen size: 659 AA
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	Lot specific
Buffer:	Liquid. Purified antibody supplied in 1x PBS buffer with 0.09 % (w/v) sodium azide and 2 % sucrose.
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Handling Advice:	Avoid repeated freeze-thaw cycles.
Storage:	-20 °C
Storage Comment:	For short term use, store at 2-8°C up to 1 week. For long term storage, store at -20°C in small aliquots to prevent freeze-thaw cycles.



Western Blotting

Image 1. WB Suggested Anti-RHOT1 Antibody Titration: 0.2-1 ug/ml ELISA Titer: 1:312500 Positive Control: 293T cell lysate RHOT1 is strongly supported by BioGPS gene expression data to be expressed in Human HEK293T cells

Successfully validated (Western Blotting (WB))

by Shaw Lab, Department of Biochemistry, University of Utah

Report Number: 100008

Date: Sep 12 2015

Target:	RHOT1
Method validated:	Western Blotting (WB)
Positive Control:	Purified recombinant Miro1 Isolated mitochondria from immortalized Miro1 KO MEFs expressing Myc-Miro1
Negative Control:	Purified recombinant Miro2 Isolated mitochondria from immortalized Miro1 WT MEFs Isolated mitochondria from immortalized Miro1 KO MEFs expressing Myc-Miro2
Notes:	Passed. ABIN405704 specifically recognizes Miro1 without cross-reaction with Miro2.
Primary Antibody:	ABIN405704
Secondary Antibody:	Goat-anti-rabbit IgG HRP conjugated (Sigma, A0545-1M22)
Protocol:	• Transfect immortalized Miro1-/- MEFs with Myc-Miro1-V1 or Myc-Miro2-V1 as described in PMID 25136135. • Isolate mitochondria from immortalized Miro1+/+, Miro1-/-, Miro1-/- + Myc-Miro1 and Miro1-/- + Myc-Miro2 MEFs. • Determine protein concentration and snap freeze mitochondria in liquid nitrogen. • Thaw 30 µg mitochondria per lane on ice. • Thaw 100 ng recombinant Miro1 and Miro2 per lane on ice. • Add 2x Laemmli buffer and 2% BME to samples and denature for 5 min at 95 °C. • Separte 30 µg/lane mitochondria and 100 ng/lane recombinant protein on a 12.5% SDS-PAGE for 3 h at 40mA/180V. • Semi-dry transfer to PVDF membrane for 2 h at 250 mA/15 V. • Block the membrane with TBST + 5% non-fat dry milk for 1 h at RT. • Incubate with primary ◦ rabbit anti-RHOT1 antibody (antibodies-online, ABIN405704) diluted 1:1000 in TBST + 3% BSA overnight at 4°C. ◦ rabbit anti-Myc1 antibody diluted 1:1000 in TBST + 3% BSA overnight at 4°C. ◦ rabbit anti-Cyt c antibody diluted 1:1000 in TBST + 3% BSA overnight at 4°C. • Wash membrane 3x for 5 min with TBST buffer. • Incubate with secondar goat anti-rabbit IgG HRP antibody (Sigma, A0545-1M2) diluted 1:2000 in TBST + 5% non-fat dry milk for 1 h at RT.

- Wash membrane 3x for 10 min with TBST buffer.
- Reveal protein bands on a Bio-Rad imaging system using homemade ECL prepared according to PMID 17141265.

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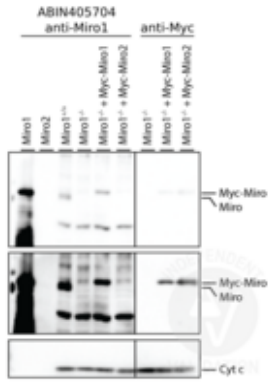
Image for Validation report #100008



Validation image no. 1 for anti-Ras Homolog Gene Family, Member T1 (RHOT1) (N-Term) antibody (ABIN405704)

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