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Datasheet for ABIN651835

anti-MKRN3 antibody (AA 160-189)

1 Validation

1 Image

Go to Product page

Overview

Quantity:	400 µL
Target:	MKRN3
Binding Specificity:	AA 160-189
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This MKRN3 antibody is un-conjugated
Application:	Western Blotting (WB)

Product Details

Immunogen:	This MKRN3 antibody is generated from rabbits immunized with a KLH conjugated synthetic peptide between 160-189 amino acids from the Central region of human MKRN3.
Clone:	RB27251
Isotype:	IgG
Purification:	This antibody is purified through a protein A column, followed by peptide affinity purification.

Target Details

Target:	MKRN3
Alternative Name:	MKRN3 (MKRN3 Products)
Background:	The protein encoded by this gene contains a RING (C3HC4) zinc finger motif and several C3H

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Target Details

	zinc finger motifs.
Molecular Weight:	55645
Gene ID:	7681
NCBI Accession:	NP_005655
UniProt:	Q13064

Application Details

Application Notes:	WB: 1:1000
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	Purified polyclonal antibody supplied in PBS with 0.09 % (W/V) sodium azide.
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:	Maintain refrigerated at 2-8 °C for up to 6 months. For long term storage store at -20 °C in small aliquots to prevent freeze-thaw cycles.
Expiry Date:	6 months

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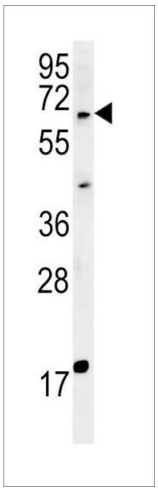

Western Blotting

Image 1. MKRN3 Antibody (Center) (ABIN651835 and ABIN2840417) western blot analysis in MDA-MB435cell line lysates (15 µg/lane).This demonstrates the MKRN3 antibody detected the MKRN3 protein (arrow).

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Successfully validated (Western Blotting (WB))

by Department of Medical Biochemistry and Microbiology, Uppsala University

Report Number: 101148

Date: Aug 10 2017

Target:	MKRN3
Lot Number:	SA100913BT
Method validated:	Western Blotting (WB)
Positive Control:	H1299 cells transfected with HA-MKRN3 expression vector; the full-length MKRN3 CDS has been inserted downstream of an HA-tag in plasmid pcDNA3.1
Negative Control:	H1299 cells transfected with HA-MKRN1 or HA-MKRN2 expression plasmid; the full-length MKRN1 or MKRN2 CDS has been inserted downstream of an HA-tag in plasmid pcDNA3 Empty plasmid pcDNA3.1
Notes:	Passed. ABIN2840417 specifically recognizes MKRN3 ectopically expressed in H1299 cells lysates.
Primary Antibody:	ABIN2840417
Secondary Antibody:	IRDye 680LT donkey anti-rabbit IgG (H+L) antibody (LI-COR, 925-68023)
Protocol:	• Grow H1299 cells (human lung carcinoma cell line) in DMEM medium (ThermoFischer Scientific, 41965-039) supplemented with 10% fetal bovine serum (ThermoFischer Scientific, 10500064) and antibiotics (1X Penicillin-Streptomycin mix, 15140122, ThermoFischer Scientific), at 37°C and 7% CO_2 in 12-well plates. • Transfect H1299 cells with plasmids expressing either HA-MKRN1, HA-MKRN2, HA-MKRN3 or with an empty plasmid pcDNA3.1 (ThermoFischer Scientific, V79020) with max 2µg of plasmid using Turbofect transfection reagent (ThermoFisher Scientific, R0531) following the manufacturer´s instructions. • Lyse cells in volume RIPA buffer. • Determine total protein content of the lysates using a Bradford protein assay (Protein Assay Dye Reagent Concentrate, Bio-Rad, 5000006). • Denature 80µg of total protein for 5min at 95°C in 20µl of 2x Laemmli SDS sample buffer and subsequently separate them on a denaturing Any kD Mini-PROTEAN TGX precast protein gel (Bio-Rad, 4569033) in Mini-PROTEAN Electrophoresis Cells for 1h at 180V. • Transfer proteins onto Amersham Protran 0.45µm nitrocellulose filter (GE Healthcare Life Sciences) with transfer buffer (25mM Tris, 192mM glycine, 20% methanol) in a Western blotting system for at 4°C for 3h at 120mA.

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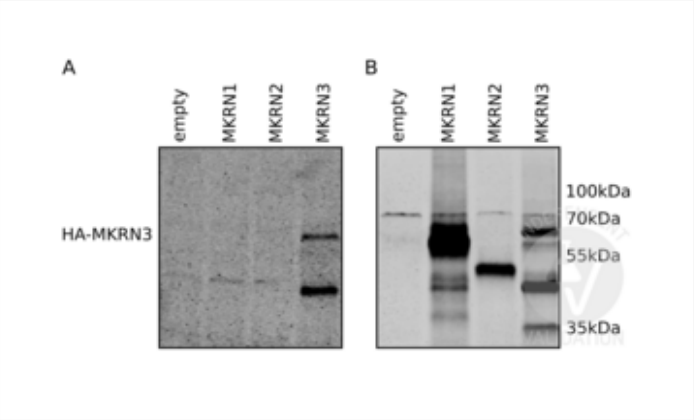
- Block the membrane with Odyssey Blocking Buffer (LI-COR, 927-50100) for 15min at RT.
- Incubation with primary
  - rabbit anti-MKRN3 antibody (antibodies-online, ABIN2840417, lot SA100913BT) diluted 1:1000 in Odyssey Blocking Buffer ON at 4°C or
  - mouse anti-HA-tag antibody (BioLegend, 901501) diluted 1:2000 in Odyssey Blocking Buffer ON at 4°C.
- Wash membrane 5x for time with PBS supplemented with 0.1% Tween 20.
- Incubation with secondary
  - IRDye 680LT donkey anti-rabbit IgG (H+L) antibody (LI-COR, 925-68023) or
  - IRDye 800CW donkey anti-mouse IgG (H+L) antibody (LI-COR, 926-32212)
- diluted 1:10000 in Odyssey Blocking Buffer for 30 min at RT.
- Wash membrane 5x for time with PBS supplemented with 0.1% Tween 20.
- Scan the membrane with LI-COR Odyssey CLX Scanner.

Experimental Notes:

- ABIN2840417 is specific towards the expressed HA-MKRN3: it does not recognize HA-MKRN1 (which has 48% identity with MKRN3) and HA-MKRN2 (has 37% identity with MKRN3) ectopically expressed in H1299 cells.
- An extraneous band can be detected with the antibody around 40kDa. This band is recognized with both anti HA- and anti MKRN3-antibodies. This suggests either C-terminal degradation product of HA-MKRN3 or that HA-MKRN3 mRNA has been not efficiently translated.
- A smear is visible for HA-MKRN3 protein with both antibodies, which may indicate that the protein undergoes self-ubiquitination or forms higher order structures.
- At present we have had difficulties to use the antibody to detect endogenous MKRN3 protein. This is probably due to the fact that endogenous MKRN3 protein levels can be very low is some cell lines.

Image for Validation report #101148



Validation image no. 1 for anti-Makorin Ring Finger Protein 3 (MKRN3) (AA 160-189) antibody (ABIN2840417)

A. Western blot of cell lysates of H1299 human lung carcinoma cells transfected with an empty, HA-tagged MKRN1, MKRN2, or MKRN3 expression plasmids (from left) using primary antibody ABIN2840417. B. Western blot analysis of the same lysates using an HA-tag specific antibody.