Datasheet for ABIN2346690

anti-RAB11A antibody (C-Term)

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1 Validation

Overview

Quantity:	100 µg
Target:	RAB11A
Binding Specificity:	C-Term
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Conjugate:	This RAB11A antibody is un-conjugated
Application:	Western Blotting (WB), ELISA, Immunohistochemistry (IHC), Immunofluorescence (IF), Immunoprecipitation (IP)

Product Details

Immunogen:	Synthetic peptide corresponding to the C-terminal hypervariable region of the human Rab11a sequence. Sequence Homology: Rab11b protein-10/17.
Isotype:	IgG
Cross-Reactivity:	Dog (Canine), Human, Rabbit, Rat (Rattus)
Cross-Reactivity (Details):	Calculated cross reactivity: Ca Hu Rb Rt
Characteristics:	Rab11A (RAB11A Member RAS Oncogene Family, Rab-11A, MGC1490, RAB11, Rab-11, Ras-related Protein Rab-11A, Ras-related protein Rab11A, YL8)
Purification:	Purified by immunoaffinity chromatography.

Target Details

Target:	RAB11A
Alternative Name:	Rab11A (RAB11A Products)
Pathways:	Regulation of Cell Size, Thromboxane A2 Receptor Signaling, Regulation of long-term Neuronal Synaptic Plasticity

Application Details

Application Notes:	Optimal working conditions should be determined by the investigator.
Restrictions:	For Research Use only

Handling

Format:	Liquid
Buffer:	Supplied as a liquid in PBS, pH 7.4, 0.09 % 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:	-20 °C
Storage Comment:	-20°C

Successfully validated (Western Blotting (WB))

by Epidermis Differentiation and Rheumatoid Autoimmunity (INSERM U1056 UDEAR), University of Toulouse

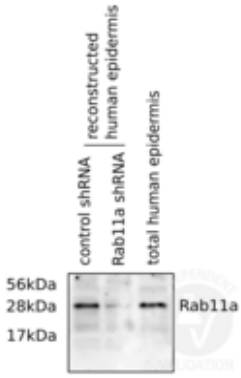
Report Number: 101022

Date: Jul 27 2017

Target:	RAB11A
Lot Number:	L16102562
Method validated:	Western Blotting (WB)
Positive Control:	Cell lysates from total human epidermis Cell lysates from reconstructed human epidermis transfected with control shRNA
Negative Control:	Cell lysates from reconstructed human epidermis transfected with RAB11A shRNA
Notes:	Passed. ABIN2346690 specifically recognizes Rab11a in human epidermal samples.
Primary Antibody:	ABIN2346690
Secondary Antibody:	goat anti-rabbit Ig HRP-conjugated antibody (Southern Biotech, 4030-05, lot J1810)
Protocol:	• Obtain total human epidermis from abdominal dermolipectomy and treat with dispase (Sigma-Aldrich, D4693) to dissociate it from the dermis. • For production of reconstructed human epidermis, obtain isolated keratinocytes from epidermal fragments. Grow cells in Epilife (Invitrogen Life Technologies, MEPICF500, lot 1838688) supplemented with penicillin and streptomycin (Sigma-Aldrich, P4333, lot 1868847), HKGS (Human Keratinocyte Growth Supplement, ThermoFisher Scientific, S0015, lot 1848268), 1.5mM calcium (Sigma-Aldrich 25011), KGF (Keratinocyte Growth Factor, Sigma-Aldrich, K1767, lot MKBX7825V) and vitamin C (Sigma-Aldrich, 49752) on polycarbonate culture inserts (Merck Millipore, PIHP01250) at 37°C and 5% CO_2, according to Reynier et al. (2016). • For silencing with interfering shRNA, transduce keratinocytes with MISSION pLKO.1 puro vector-based lentiviral particles containing a puromycin resistance gene and an shRNA insert under the human U6 promoter (Sigma-Aldrich, TRCN0000303291 for Rab11A and SHC002 for the negative control) following the manufacturer´s instructions, before cell seeding for production of reconstructed human epidermis. • Lyse normal or reconstructed epidermis in 300µL/0.6cm^2 1x Laemmli buffer (Tris-HCl 63mM pH6.8, SDS 2%, Glycerol 10%, Bromophenol blue 0.0005%, DTT 1mM, all Sigma-Aldrich) and heat at 95°C for 5min. • Separate 5µL of total protein extracts in 1x Laemmli buffer on denaturing 10% polyacrylamide SDS gels in a Mini-Protean Tetra Cell electrophoresis cell system (Bio-Rad , 1658004) at 100V for 90min.

- Transfer proteins onto Hybond ECL membrane (GE Healthcare, RPN3032D) with a Mini TransBlot Western blotting system (Bio-Rad, 1658029) at 100V for 90 min.
- Block membrane with 1x PBS containing Tween 20 (Sigma-Aldrich) at 0.05% (PBST) and 5% low fat milk at 3% at RT for 1h.
- Incubate the membrane with primary rabbit anti-human RAB11A, Member RAS Oncogene Family (RAB11A) (C-Term) antibody (antibodies-online, ABIN2346690, lot L16102562) diluted 1:250 in blocking buffer ON at 4°C.
- Wash the membrane 3x for 15min with 1x PBST.
- Incubate the membrane with secondary goat anti-rabbit Ig HRP-conjugated antibody (Southern Biotech, 4030-05, lot J1810) diluted 1:10000 in blocking buffer for 90min at RT.
- Wash the membrane 3x for 15min with 1x PBST.
- Reveal protein bands using ECL prime Detection Reagent (GE Healthcare, RPN2238, lot 9838391) on a GBox apparatus (Syngene).

Image for Validation report #101022



Validation image no. 1 for anti-RAB11A, Member RAS Oncogene Family (RAB11A) (C-Term) antibody (ABIN2346690)

Western blot analysis of reconstructed human epidermis cell lysates with ABIN2346690 reveals a band of the expected size for Rab11a in the lysates of the cells transfected with a scrambled control shRNA (left lane) which is absent in lysates from cells that were transfected with RAB11A shRNA. Total human epidermis lysates (right lane) served as control. The immunoblot with ABIN2346690 reveals the expected protein band.