abcam

Product datasheet

Anti-RAB7 antibody [EPR7589] ab137029

KO VALIDATED Recombinant RabMAb

★★★★★ 23 Abreviews 92 References 11 Images

Overview

Product name	Anti-RAB7 antibody [EPR7589]
Description	Rabbit monoclonal [EPR7589] to RAB7
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Wild-type HAP1 cell lysate; HeLa, A375, A431, U87-MG, HT1080, L929, NIH/3T3, Raw 264.7 and C2C12 cell lysates. IHC-P: Human kidney tissue. ICC/IF: HeLa, NIH/3T3 and HepaRG cells. Flow Cyt (intra): HAP1-WT cells; A431 cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.31% Sodium citrate, 0.175% Sodium chloride, 0.0172% EDTA, 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR7589
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab137029 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/10 - 1/100. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (12)	1/1000 - 1/10000. Predicted molecular weight: 23 kDa.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★★ (8)	1/100 - 1/500.

Application notes Is unsuitable for IP.

Target

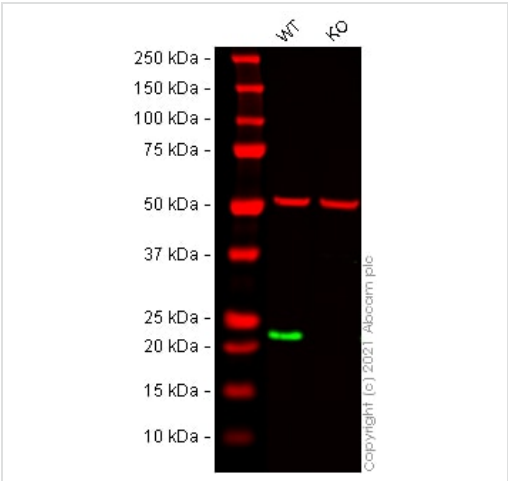
Function Key regulator in endo-lysosomal trafficking. Governs early-to-late endosomal maturation, microtubule minus-end as well as plus-end directed endosomal migration and positioning, and endosome-lysosome transport through different protein-protein interaction cascades. Plays a central role, not only in endosomal traffic, but also in many other cellular and physiological events, such as growth-factor-mediated cell signaling, nutrient-transportor mediated nutrient uptake, neurotrophin transport in the axons of neurons and lipid metabolism. Also involved in regulation of some specialized endosomal membrane trafficking, such as maturation of melanosomes, pathogen-induced phagosomes (or vacuoles) and autophagosomes. Plays a role in the maturation and acidification of phagosomes that engulf pathogens, such as S.aureus and M.tuberculosis. Plays a role in the fusion of phagosomes with lysosomes. Plays important roles in microbial pathogen infection and survival, as well as in participating in the life cycle of viruses. Microbial pathogens possess survival strategies governed by RAB7A, sometimes by employing RAB7A function (e.g. Salmonella) and sometimes by excluding RAB7A function (e.g. Mycobacterium). In concert with RAC1, plays a role in regulating the formation of RBs (ruffled borders) in osteoclasts. Controls the endosomal trafficking and neurite outgrowth signaling of NTRK1/TRKA. Regulates the endocytic trafficking of the EGF-EGFR complex by regulating its lysosomal degradation.

Tissue specificity Widely expressed; high expression found in skeletal muscle.

Involvement in disease Defects in RAB7A are the cause of Charcot-Marie-Tooth disease type 2B (CMT2B) [MIM:600882]; also known as hereditary motor and sensory neuropathy II (HMSN2). CMT2B is a form of Charcot-Marie-Tooth disease, the most common inherited disorder of the peripheral nervous system. Charcot-Marie-Tooth disease is classified in two main groups on the basis of electrophysiologic properties and histopathology: primary peripheral demyelinating neuropathy or CMT1, and primary peripheral axonal neuropathy or CMT2. Neuropathies of the CMT2 group are

	characterized by signs of axonal regeneration in the absence of obvious myelin alterations, normal or slightly reduced nerve conduction velocities, and progressive distal muscle weakness and atrophy. CMT2B is clinically characterized by marked distal muscle weakness and a high frequency of foot ulcers, infections and amputations of the toes. CMT2B inheritance is autosomal dominant.
Sequence similarities	Belongs to the small GTPase superfamily. Rab family.
Cellular localization	Late endosome. Lysosome. Cytoplasmic vesicle > phagosome. Melanosome. Cytoplasmic vesicle > phagosome membrane. Co-localizes with OSBPL1A at the late endosome. Found in the ruffled border (a late endosomal-like compartment in the plasma membrane) of bone-resorbing osteoclasts. Recruited to phagosomes containing S.aureus or Mycobacterium.

Images



Western blot - Anti-RAB7 antibody [EPR7589] (ab137029)

All lanes : Anti-RAB7 antibody [EPR7589] (ab137029) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RAB7A knockout HeLa cell lysate

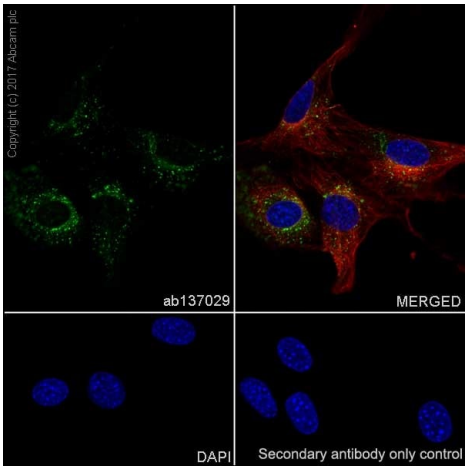
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 23 kDa
Observed band size: 23 kDa

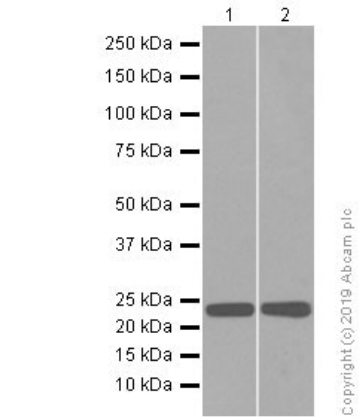
Lanes 1 - 2: Merged signal (red and green). Green - ab137029 observed at 23 kDa. Red - loading control **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) observed at 55 kDa.

ab137029 was shown to react with RAB7 in wild-type HeLa cells in Western blot with loss of signal observed in RAB7A knockout cell line **ab255423** (RAB7A knockout cell lysate **ab263831**). Wild-type HeLa and RAB7A knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab137029 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-RAB7 antibody [EPR7589] (ab137029)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% tritonX-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labelling RAB7 with ab137029 at 1:250 dilution, followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1:1000 dilution (Green). Confocal image showing cytoplasmic staining on NIH/3T3 cell line is observed. Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1:200 dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1:1000 dilution.



Western blot - Anti-RAB7 antibody [EPR7589] (ab137029)

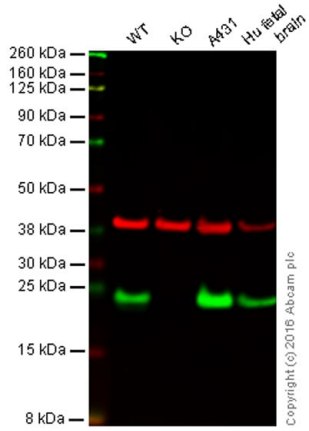
All lanes : Anti-RAB7 antibody [EPR7589] (ab137029) at 1/1000 dilution

Lane 1 : A375 (Human malignant melanoma epithelial cell) whole cell lysates
Lane 2 : HT-1080 (Human fibrosarcoma epithelial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

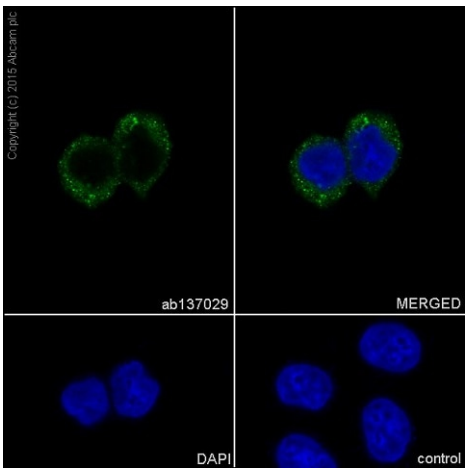
Predicted band size: 23 kDa
Observed band size: 23 kDa



Western blot - Anti-RAB7 antibody [EPR7589] (ab137029)

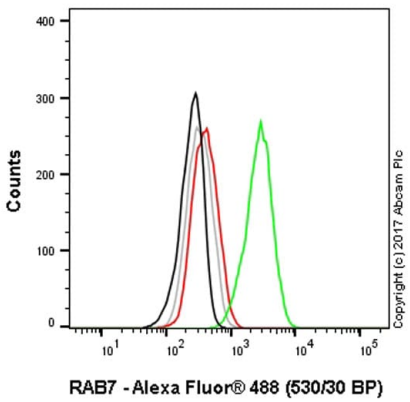
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: RAB7 knockout HAP1 cell lysate (20 µg)
Lane 3: A431 cell lysate (20 µg)
Lane 4: Human fetal brain tissue lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab137029 observed at 24 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab137029 was shown to specifically react with RAB7 in wild-type HAP1 cells. No band was observed when RAB7 knockout samples were examined. Wild-type and RAB7 knockout samples were subjected to SDS-PAGE. ab137029 and **ab8245** (loading control to GAPDH) were both diluted 1/1000 and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10 000 dilution for 1 hour at room temperature before imaging.



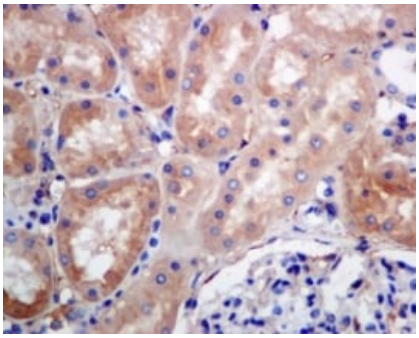
Immunocytochemistry/ Immunofluorescence - Anti-RAB7 antibody [EPR7589] (ab137029)

Immunocytochemistry/Immunofluorescence analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) labelling RAB7 with purified ab137029 at 1/500. Cells were fixed with 100% methanol. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).



Flow Cytometry (Intracellular) - Anti-RAB7 antibody [EPR7589] (ab137029)

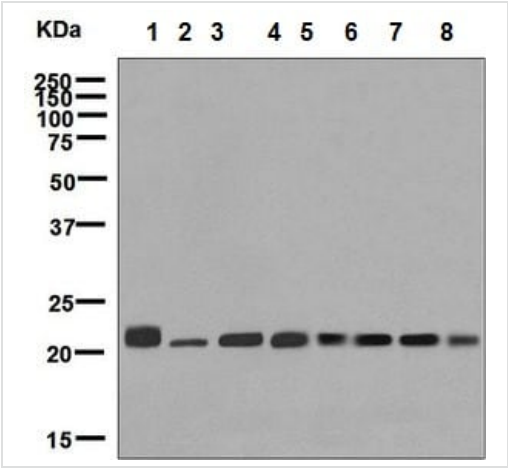
Overlay histogram showing HAP1 wildtype (green line) and HAP1-RAB7 knockout cells (red line) stained with ab137029. The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab137029, 0.1µg/ml) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150081**) at 1/2000 dilution for 30 min at 22°C. A rabbit IgG1 isotype control antibody (**ab172730**) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-RAB7 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity). Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter. This antibody can also be used in HAP1 cells fixed with 80% methanol (5 min) permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAB7 antibody [EPR7589] (ab137029)

Immunohistochemical analysis of paraffin embedded Human kidney tissue labelling RAB7 with ab137029 at 1/50 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Western blot - Anti-RAB7 antibody [EPR7589] (ab137029)

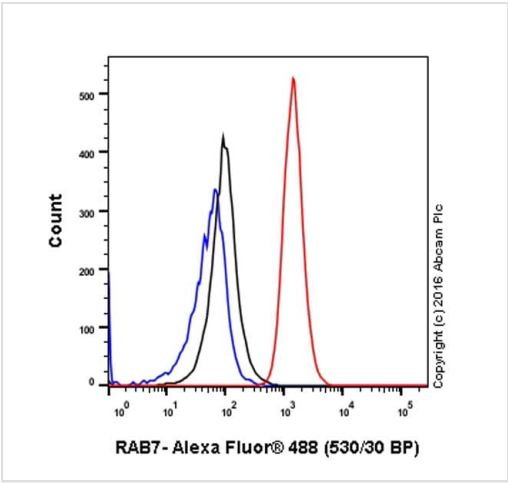
All lanes : Anti-RAB7 antibody [EPR7589] (ab137029) at 1/1000 dilution

Lane 1 : A375 (human malignant melanoma cell line) cell lysate
Lane 2 : A431 (human epidermoid carcinoma cell line) cell lysate
Lane 3 : U87 MG (human glioblastoma-astrocytoma epithelial cell line) cell lysate
Lane 4 : HT 1080 (human fibrosarcoma cell line) cell lysate
Lane 5 : L929 (mouse connective tissue fibroblast cell line) cell lysate
Lane 6 : NIH 3T3 (mouse embyro fibroblast cell line) cell lysate
Lane 7 : Raw 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cell lysate
Lane 8 : C2C12 (mouse myoblast cell line) cell lysate

Lysates/proteins at 10 µg per lane.

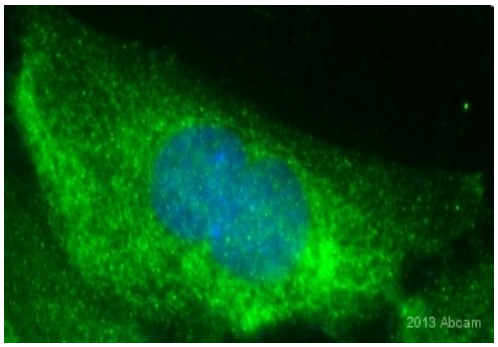
Secondary
All lanes : HRP conjugated Goat anti Rabbit IgG at 1/2000 dilution

Predicted band size: 23 kDa



Flow Cytometry (Intracellular) - Anti-RAB7 antibody [EPR7589] (ab137029)

Intracellular Flow Cytometry analysis of A431 (human epidermoid carcinoma cell line) cells labeling RAB7 with purified ab137029 at 1/20 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



Immunocytochemistry/ Immunofluorescence - Anti-RAB7 antibody [EPR7589] (ab137029)

This image is courtesy of an Abreview submitted by Alina Macovei

ab137029 staining RAB7 in Human HepaRG cell by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with formaldehyde, permeabilized with 0.1% Triton X-100 in PBS and blocked with 1% milk for 30 minutes at room temperature. Samples were incubated with primary antibody (1/200 in 1% milk) for 30 minutes. An Alexa Fluor®488-conjugated Donkey anti-rabbit IgG polyclonal (1/400) was used as the secondary antibody.

Anti-RAB7 antibody [EPR7589] (ab137029)

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