abcam

Product datasheet

Anti-Rab4 antibody [EPR3042] - Early Endosome Marker ab108974

KO VALIDATED Recombinant RabMAb

4 References 7 Images

Overview

Product name	Anti-Rab4 antibody [EPR3042] - Early Endosome Marker
Description	Rabbit monoclonal [EPR3042] to Rab4 - Early Endosome Marker
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF **Unsuitable for:** Flow Cyt or IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	HeLa, MCF7, PC12, Neuro-2a, mouse brain, rat brain and fetal brain cell lysates
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 +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle. Stable for 12 months at -20°C.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR3042

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab108974 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
WB		1/2000 - 1/10000. Detects a band of approximately 25 kDa (predicted molecular weight: 24 kDa).
ICC/IF		1/100 - 1/250.

Application notes Is unsuitable for Flow Cyt or IHC-P.

Target

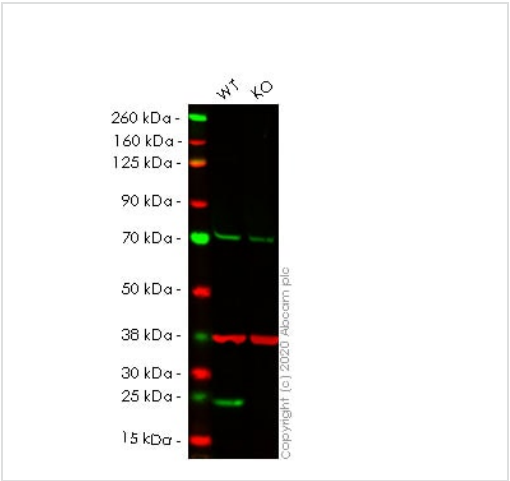
Function Protein transport. Probably involved in vesicular traffic.

Sequence similarities Belongs to the small GTPase superfamily. Rab family.

Post-translational modifications Phosphorylated by CDK1 kinase during mitosis.

Cellular localization Membrane. Cytoplasm. Generally associated with membranes. Cytoplasmic when phosphorylated by CDK1.

Images



Western blot - Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974)

All lanes : Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RAB4A knockout HeLa cell lysate

Lysates/proteins at 40 µg per lane.

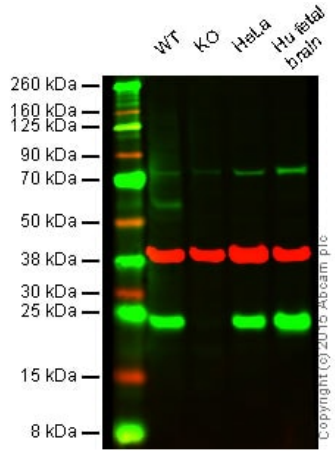
Performed under reducing conditions.

Predicted band size: 24 kDa
Observed band size: 24 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab108974 observed at 24 kDa. Red - Anti-GAPDH antibody [6C5] - Loading

Control (**ab8245**) observed at 37 kDa.

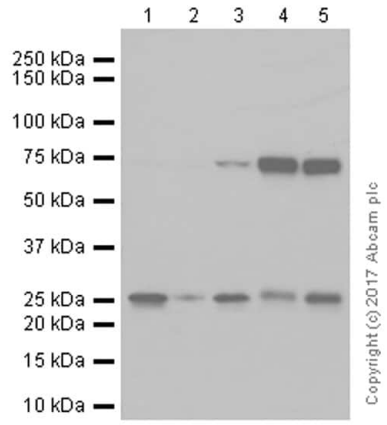
ab108974 was shown to react with Rab4 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab264781** (knockout cell lysate **ab257624**) was used. Wild-type HeLa and RAB4A knockout HeLa cell lysates were subjected to SDS-PAGE. ab108974 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. 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 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: Rab4 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Human fetal brain cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab108974 observed at 24 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

Unpurified ab108974 was shown to recognize Rab4 when Rab4 knockout samples were used, along with additional cross-reactive bands. Wild-type and Rab4 knockout samples were subjected to SDS-PAGE. Unpurified ab108974 and **ab8245** (loading control to GAPDH) were both diluted 1/2000 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 h at room temperature before imaging.



Western blot - Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974)

All lanes : Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974) at 1/2000 dilution (purified)

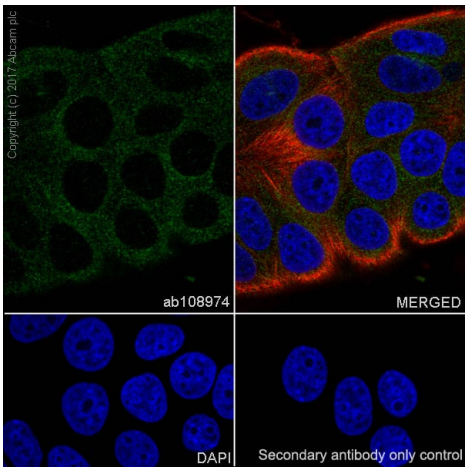
Lane 1 : MCF7 (Human breast adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates
Lane 3 : Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysates
Lane 4 : Mouse brain lysates
Lane 5 : Rat brain lysates

Lysates/proteins at 20 µg per lane.

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

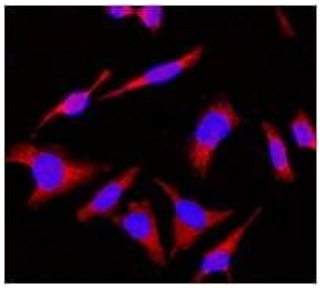
Predicted band size: 24 kDa
Observed band size: 25 kDa

Blocking and diluting buffer: 5% NFDM/TBST



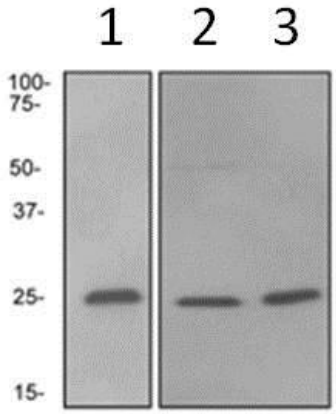
Immunocytochemistry/ Immunofluorescence - Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974)

Immunocytochemistry/ Immunofluorescence analysis of MCF7 (Human breast adenocarcinoma epithelial cell) cells labeling Rab4 with purified ab108974 at 1:100 dilution (8.8µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Immunocytochemistry/ Immunofluorescence - Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974)

Immunofluorescent staining of Rab4 in HeLa cells, using unpurified ab108974 at a 1/100 dilution.



Western blot - Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974)

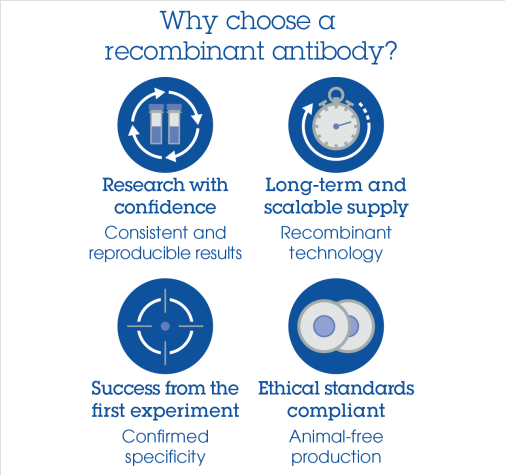
All lanes : Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974) at 1/2000 dilution (unpurified)

Lane 1 : MCF7 cell lysates
Lane 2 : PC12 cell lysates
Lane 3 : Fetal brain cell lysates

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 24 kDa
Observed band size: 25 kDa



Anti-Rab4 antibody [EPR3042] - Early Endosome Marker (ab108974)

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