abcam

Product datasheet

Anti-RAB8A antibody [MJF-R22-79-3] ab241061

KO VALIDATED Recombinant RabMAb

10 Images

Overview

Product name	Anti-RAB8A antibody [MJF-R22-79-3]
Description	Rabbit monoclonal [MJF-R22-79-3] to RAB8A
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Human
Immunogen	Recombinant full length protein within Human RAB8A aa 1 to the C-terminus. The exact immunogen sequence used to generate this antibody is proprietary information. If additional detail on the immunogen is needed to determine the suitability of the antibody for your needs, please **contact** our Scientific Support team to discuss your requirements. Database link: **P61006** Run BLAST with Run BLAST with
Positive control	WB: HEK-293T, HCT116 and HeLa whole cell lysates. IHC-P: Human bladder cancer and breast tissues. ICC/IF: A549 and HeLa cells. Flow Cyt (intra): A549 cells. IP: A549 whole cell lysate.
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**. This antibody was developed with support from The Michael J. Fox Foundation. THE MICHAEL J. FOX FOUNDATION FOR PARKINSON'S RESEARCH

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.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	MJF-R22-79-3
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab241061 in the following tested applications.

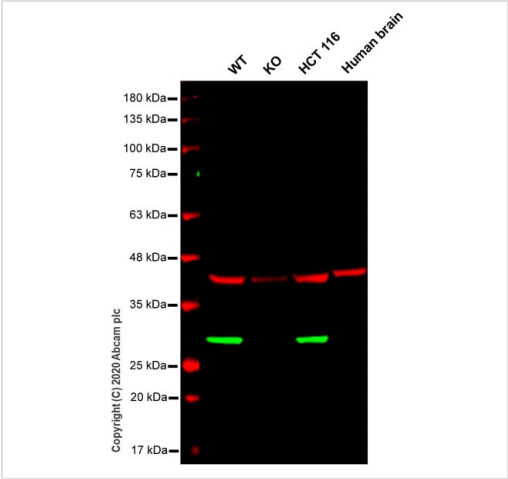
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/600.
WB		1/1000. Detects a band of approximately 23 kDa (predicted molecular weight: 24 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.
IP		1/30.

Target

Function	May be involved in vesicular trafficking and neurotransmitter release. Together with RAB11A, RAB3IP, the exocyst complex, PARD3, PRKCI, ANXA2, CDC42 and DNMBP promotes transcytosis of PODXL to the apical membrane initiation sites (AMIS), apical surface formation and lumenogenesis. Together with MYO5B and RAB11A participates in epithelial cell polarization.
Sequence similarities	Belongs to the small GTPase superfamily. Rab family.
Cellular localization	Cell membrane. Golgi apparatus. Cytoplasm > perinuclear region. Cell projection. Colocalizes with OPTN at the Golgi complex and in vesicular structures close to the plasma membrane. In the GDP-bound form, present in the perinuclear region. Shows a polarized distribution to distal regions of cell protrusions in the GTP-bound form. Colocalizes with PARD3, PRKCI, EXOC5, OCLN, PODXL and RAB11A in apical membrane initiation sites (AMIS) during the generation of apical surface and luminogenesis.

Images



Western blot - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

All lanes : Anti-RAB8A antibody [MJF-R22-79-3] (ab241061) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RAB8A knockout HeLa cell lysate
Lane 3 : HCT116 cell lysate
Lane 4 : Human brain tissue lysate

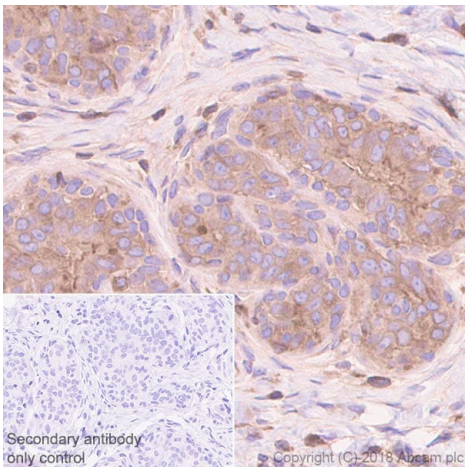
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1-4: Merged signal (red and green). Green - ab241061 observed at 24 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab241061 Anti-RAB8A antibody [MJF-R22-79-3] was shown to specifically react with RAB8A in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab264993** (knockout cell lysate **ab257195**) was used. Wild-type and RAB8A knockout samples were subjected to SDS-PAGE. ab ab241061 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 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 10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

Immunohistochemical analysis of paraffin-embedded human breast tissue labeling RAB8A with ab241061 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on human breast (PMID: 14581456). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Western blot - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

All lanes : Anti-RAB8A antibody [MJF-R22-79-3] (ab241061) at 1/1000 dilution

Lane 1 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

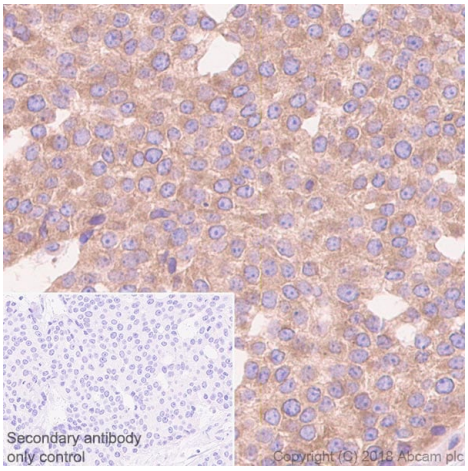
Lysates/proteins at 20 µg per lane.

Predicted band size: 24 kDa

Exposure time : 7.75 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

The expression profile observed is consistent with what has been described in the literature (PMID: 15673612).

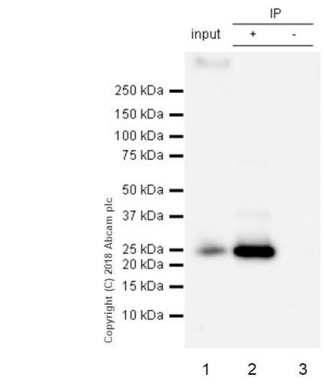


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

Immunohistochemical analysis of paraffin-embedded human bladder cancer tissue labeling RAB8A with ab241061 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on human bladder cancer (PMID: 14581456). Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunoprecipitation - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

RAB8A was immunoprecipitated from 0.35 mg of A549 (human lung carcinoma cell line) whole cell lysate with ab241061 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab241061 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/5000 dilution.

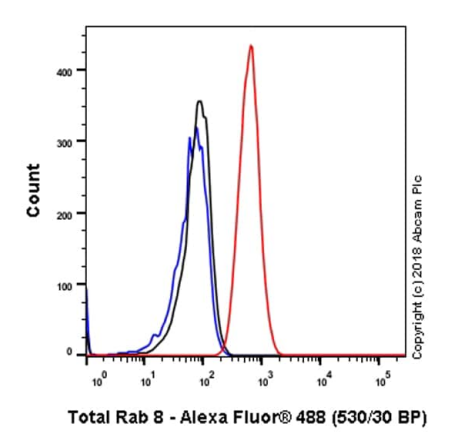
Lane 1: A549 whole cell lysate 10 µg (Input).

Lane 2: ab241061 IP in A549 whole cell lysate .

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab241061 in A549 whole cell lysate .

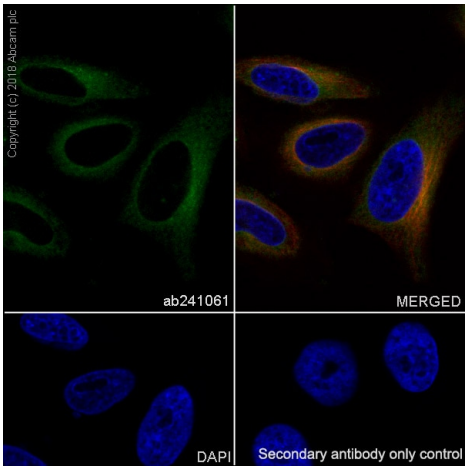
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 8 seconds.



Flow Cytometry (Intracellular) - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized A549 (human lung carcinoma cell line) cell line labeling RAB8A with ab241061 at 1/600 dilution (red) compared with a Rabbit IgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/2000 dilution was used as the secondary antibody.

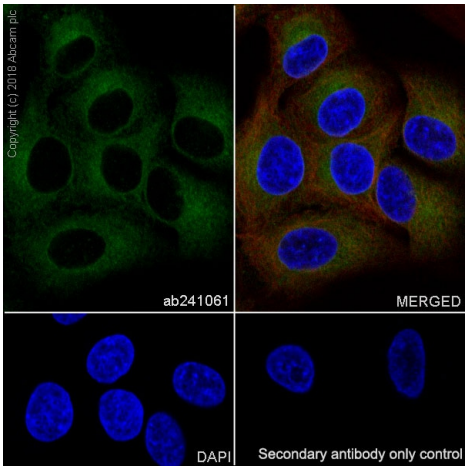


Immunocytochemistry/ Immunofluorescence - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling RAB8A with ab241061 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in HeLa cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.

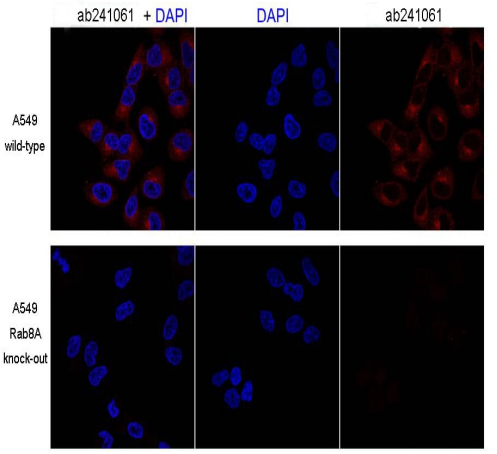


Immunocytochemistry/ Immunofluorescence - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized A549 (human lung carcinoma cell line) cells labeling RAB8A with ab241061 at 1/500 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining in A549 cells.

The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

Immunofluorescent analysis of 3% paraformaldehyde-fixed, 0.1% Saponin permeabilized A549 (human lung carcinoma cell line) and RAB8A knock-out A549 cells labeling RAB8A with ab241061 at 1/100 dilution.

This image is kindly provided by our collaborator Dr. Dario Alessi, University of Dundee.

Anti-RAB8A antibody [MJF-R22-79-3] (ab241061)

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