abcam

Product datasheet

Anti-Rab5 antibody [EPR21801] - Early Endosome Marker ab218624

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Rab5 antibody [EPR21801] - Early Endosome Marker
Description	Rabbit monoclonal [EPR21801] to Rab5 - Early Endosome Marker
Host species	Rabbit
Specificity	This antibody could cross-react with RAB5C, but affinity is very low.
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, MCF7 and Neuro-2a whole cell lysates; Human fetal brain, fetal heart and fetal spleen lysates; Rat and mouse brain and heart lysates. IHC-P: Human kidney and cerebrum tissues; Mouse kidney tissue; Rat liver tissue. ICC/IF: NIH/3T3, HeLa and HAP1 cells. Flow Cyt (intra): MCF7 cells, Hap1 cells. IP: MCF7 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**.

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, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR21801
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab218624 in the following tested applications.

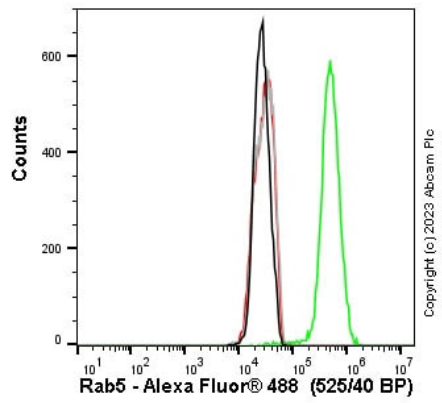
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/50.
WB		1/1000. Detects a band of approximately 25 kDa (predicted molecular weight: 24 kDa).
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/1000.
IP		1/30.

Target

Function	Required for the fusion of plasma membranes and early endosomes.
Sequence similarities	Belongs to the small GTPase superfamily. Rab family.
Cellular localization	Cell membrane. Early endosome membrane. Melanosome. Enriched in stage I melanosomes.

Images



Flow Cytometry (Intracellular) - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

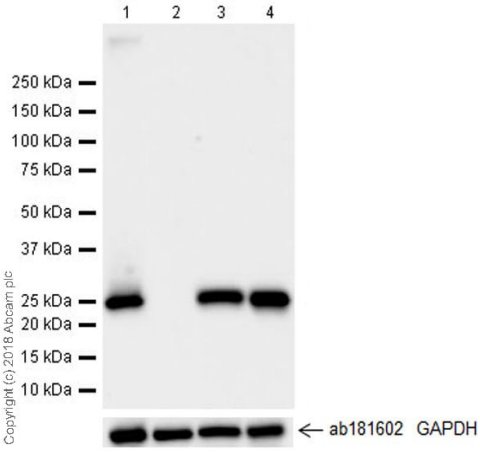
Flow cytometry overlay histogram showing wild-type Hap1 (green line) and Rab5A knockout Hap1 stained with ab218624 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (ab218624) (1x 10^6 in 100µl at 0.2 µg/ml (1/10700)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control was used at the same concentration and conditions as the primary antibody (wild-type Hap1 - black line, Rab5A knockout Hap1 - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.

This antibody gave a positive signal in Hap1 Fixed with 4% formaldehyde (10 min) / permeabilised with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Western blot - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

All lanes : Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624) at 1/1000 dilution

Lane 1 : Wild type HAP1 whole cell lysate
Lane 2 : Rab5 knockout HAP1 whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 4 : MCF7 (Human breast adenocarcinoma cell line) whole cell lysate

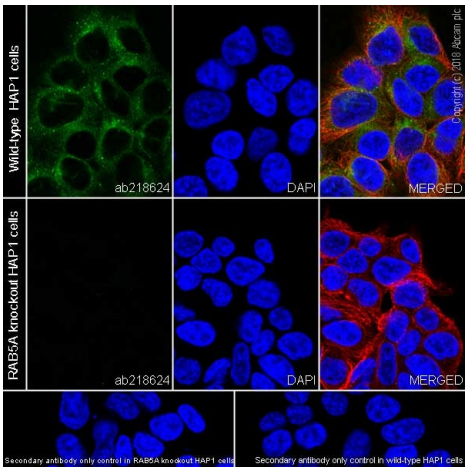
Lysates/proteins at 20 µg per lane.

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

Exposure time: 15 seconds

ab218624 was shown to specifically react with Rab5 in wild-type

HAP1 cells as signal was lost in Rab5 knockout cells. Wild-type and Rab5 knockout samples were subjected to SDS-PAGE. Ab218624 and **ab181602** (Human anti-GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/200000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD® ChemiDoc™ MP instrumentusing the ECL technique.

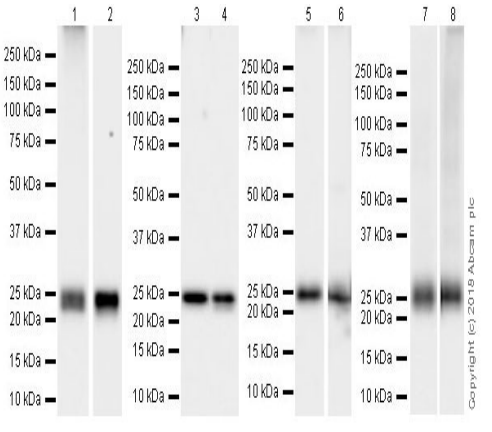


Immunocytochemistry/ Immunofluorescence - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilzed wild-type and RAB5A KO HAP1 cells labeling Rab5 with ab218624 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing no staining in RAB5A KO HAP1 cell line and granular cytoplasmic staining in parental HAP1 cell line.

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.



Western blot - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

All lanes : Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624) at 1/1000 dilution

Lane 1 : Neuro-2a (mouse neuroblastoma cell line) whole cell lysate
Lane 2 : Human fetal brain lysate
Lane 3 : Human fetal heart lysate
Lane 4 : Human fetal spleen lysate
Lane 5 : Rat brain lysate
Lane 6 : Rat heart lysate
Lane 7 : Mouse brain lysate
Lane 8 : Mouse heart lysate

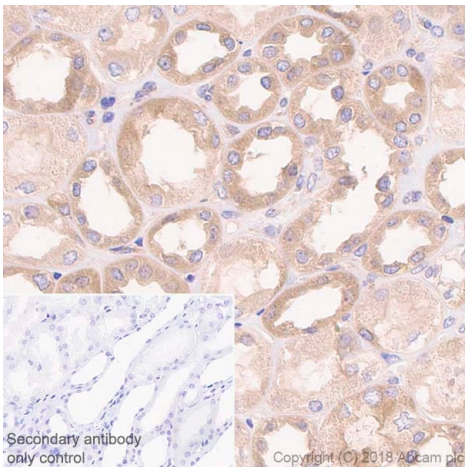
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time : Lane 1: 5 seconds; Lanes 2/8: 15 seconds; Lanes 3/4: 6 seconds; Lanes 5/7: 3 seconds; Lane 6: 26 seconds.

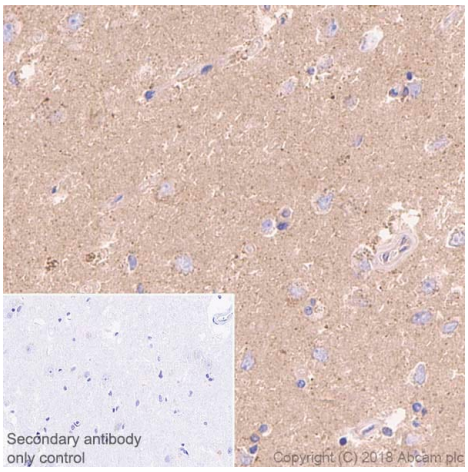
Blocking/Dilution buffer: 5% NFDM/TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling Rab5 with ab218624 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining in human kidney is observed (PMID 7789520). Counterstained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

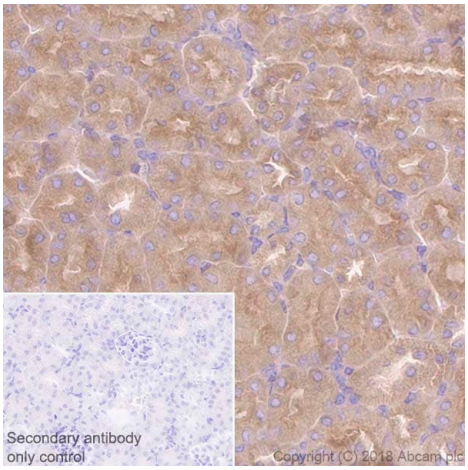
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling Rab5 with ab218624 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining in human cerebrum is observed (PMID 7789520). Counterstained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

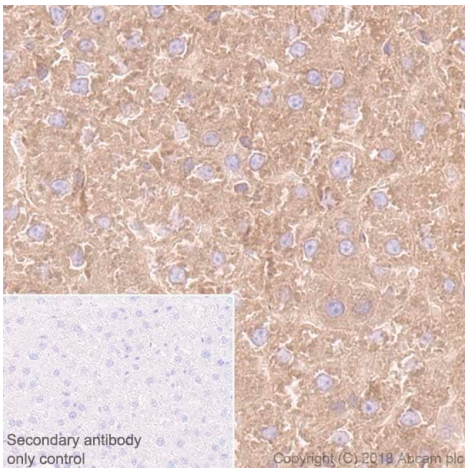
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling Rab5 with ab218624 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining in mouse kidney is observed (PMID 7789520). Counterstained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

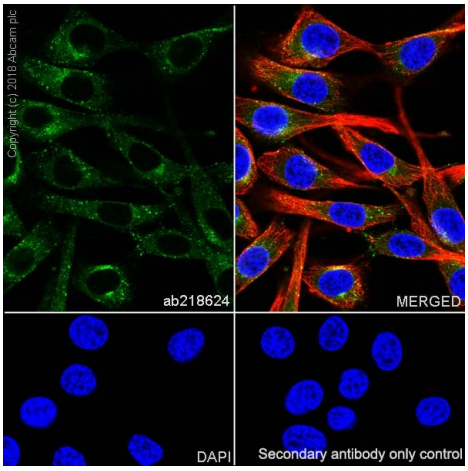
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Immunohistochemical analysis of paraffin-embedded rat liver tissue labeling Rab5 with ab218624 at 1/4000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining in rat liver is observed (PMID 7789520). Counterstained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

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

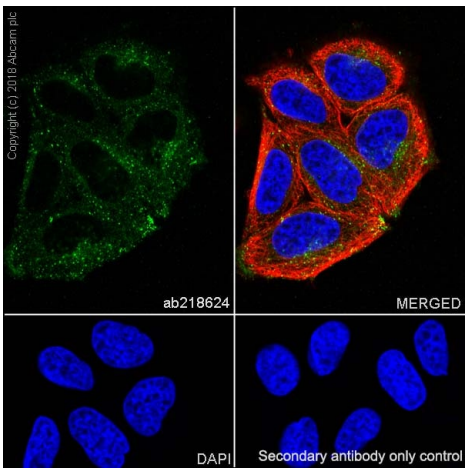


Immunocytochemistry/ Immunofluorescence - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilzed NIH/3T3 (mouse embryo fibroblast cell line) cells labeling Rab5 with ab218624 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing granular cytoplasmic staining in NIH/3T3 cell line.

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.

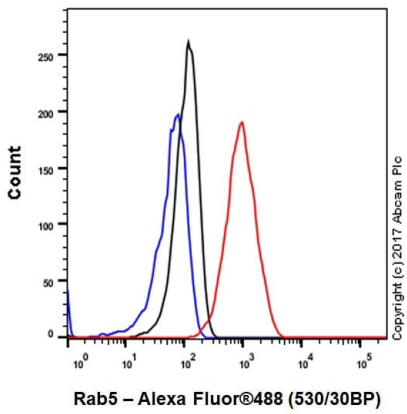


Immunocytochemistry/ Immunofluorescence - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilzed HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Rab5 with ab218624 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing granular cytoplasmic staining in HeLa cell line.

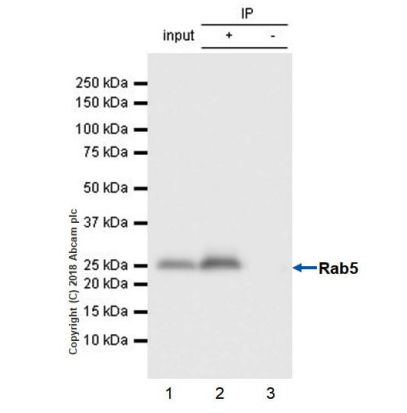
The nuclear counter stain is DAPI (blue). Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) (red) at 1/200 dilution.

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.



Flow Cytometry (Intracellular) - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized MCF7 (human breast adenocarcinoma cell line) cell line labelling Rab5 with ab218624 at 1/50 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 (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

Rab5 was immunoprecipitated from 0.35 mg MCF7 (human breast adenocarcinoma cell line) whole cell lysate with ab218624 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab218624 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used for detection at 1/5000 dilution.

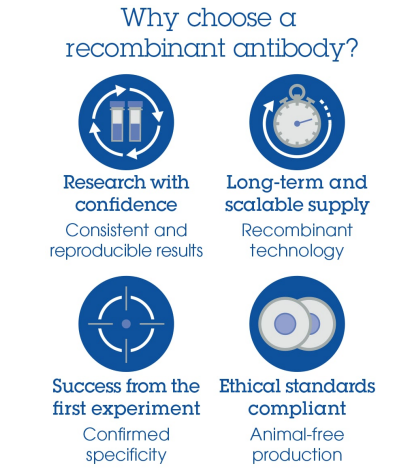
Lane 1: MCF7 whole cell lysate 10 µg (input).

Lane 2: ab218624 IP in MCF7 whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab218624 in MCF7 whole cell lysate.

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

Exposure time: 1 second.



Anti-Rab5 antibody [EPR21801] - Early Endosome Marker (ab218624)

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