abcam

Product datasheet

Anti-Rab5 antibody [EPR21801] - BSA and Azide free ab231095



יובעדער RabMAb

画像数 12

製品の概要

製品名 Anti-Rab5 antibody [EPR21801] - BSA and Azide free

製品の詳細 Rabbit monoclonal [EPR21801] to Rab5 - BSA and Azide free

由来種 Rabbit

特異性 This antibody could cross-react with RAB5C, but affinity is very low.

アプリケーション 適用あり: Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: HAP1, HeLa, and MCF7 whole cell lysates. Flow cyto(intra): HAP1 cells

特記事項 ab231095 is the carrier-free version of ab218624.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

バッファー pH: 7.2

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EPR21801

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab231095の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 25 kDa (predicted molecular weight: 24 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

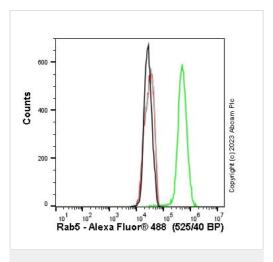
ターゲット情報

機能 Required for the fusion of plasma membranes and early endosomes.

配列類似性 Belongs to the small GTPase superfamily. Rab family.

細胞内局在 Cell membrane. Early endosome membrane. Melanosome. Enriched in stage I melanosomes.

画像



Flow Cytometry (Intracellular) - Anti-Rab5 antibody [EPR21801] - BSA and Azide free (ab231095)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).

Flow cytometry overlay histogram showing wild-type Hap1 (green line) and RAB5A knockout Hap1 stained with <u>ab218624</u> (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 (<u>ab218624</u>) (1x 10^6 in 100μ l at $0.2~\mu$ g/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.

1 2 3 4

250 kDa —
150 kDa —
150 kDa —
75 kDa —
50 kDa —
37 kDa —
37 kDa —
37 kDa —
315 kDa —
4 ab181602 GAPDH

Western blot - Anti-Rab5 antibody [EPR21801] - BSA and Azide free (ab231095)

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.

Secondary

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

Predicted band size: 24 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).

Secondary antibody only control

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Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Rab5 antibody

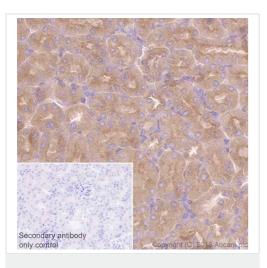
[EPR21801] - BSA and Azide free (ab231095)

Immunohistochemical analysis of paraffin-embedded rat liver tissue labeling Rab5 with <u>ab218624</u> 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.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).

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



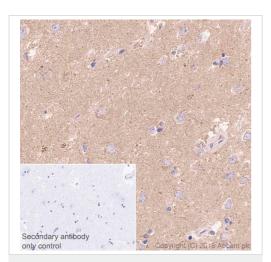
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Rab5 antibody
[EPR21801] - BSA and Azide free (ab231095)

Immunohistochemical analysis of paraffin-embedded mouse kidney tissue labeling Rab5 with <u>ab218624</u> 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.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Rab5 antibody

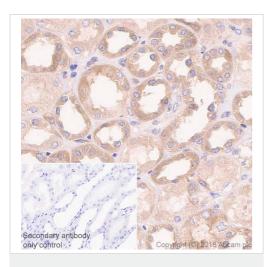
[EPR21801] - BSA and Azide free (ab231095)

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling Rab5 with <u>ab218624</u> 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.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).

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



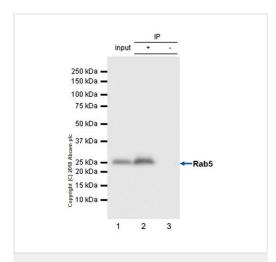
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Rab5 antibody
[EPR21801] - BSA and Azide free (ab231095)

Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling Rab5 with <u>ab218624</u> 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.

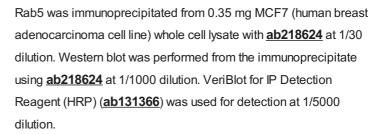
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab218624</u>).

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



Immunoprecipitation - Anti-Rab5 antibody
[EPR21801] - BSA and Azide free (ab231095)



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

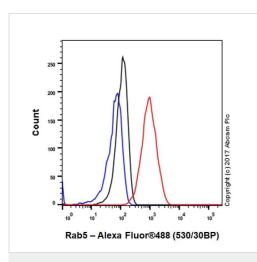
Lane 2: ab218624 IP in MCF7 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of $\underline{ab218624}$ in MCF7 whole cell lysate.

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

Exposure time: 1 second.

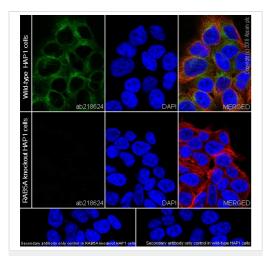
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).



Flow Cytometry (Intracellular) - Anti-Rab5 antibody [EPR21801] - BSA and Azide free (ab231095)

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 lgG, monoclonal [EPR25A] - Isotype Control (**ab172730**) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti rabbit lgG (Alexa Fluor 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).



Immunocytochemistry/ Immunofluorescence - Anti-Rab5 antibody [EPR21801] - BSA and Azide free (ab231095)

ab218624 MERGED

DAPI

Secondary antibody only control

Immunocytochemistry/ Immunofluorescence - Anti-Rab5 antibody [EPR21801] - BSA and Azide free (ab231095)

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 lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.

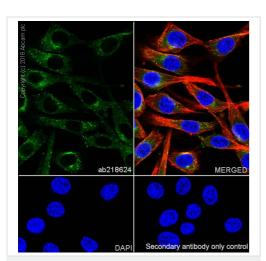
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilzed HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Rab5 with <u>ab218624</u> at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) 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 lgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab218624</u>).



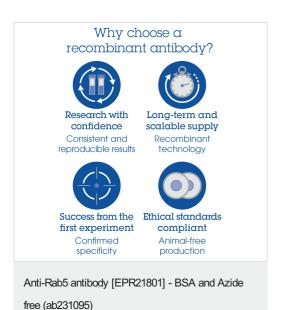
Immunocytochemistry/ Immunofluorescence - Anti-Rab5 antibody [EPR21801] - BSA and Azide free (ab231095)

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 lgG H&L (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab218624).



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