abcam

Product datasheet

Anti-RAB29 antibody [MJF-R30-104] - BSA and Azide free ab256548





RabMAb

4 Images

Overview

Product name Anti-RAB29 antibody [MJF-R30-104] - BSA and Azide free

Description Rabbit monoclonal [MJF-R30-104] to RAB29 - BSA and Azide free

Host species Rabbit

Tested applications Suitable for: WB, IP

Unsuitable for: Flow Cyt,ICC/IF or IHC-P

Species reactivity Reacts with: Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Wild type A549, A549, HEK-293T cells. HEK-293T, MCF-7, Caco-2 cells lysates. IP: A549

cells.

General notes ab256548 is the carrier-free version of <u>ab256527</u>.

Our <u>carrier-free</u> 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 cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> 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.

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

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monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

This antibody was developed with support from The Michael J. Fox Foundation.



Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C. Do Not Freeze.

Storage buffer pH: 7.2

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number MJF-R30-104

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab256548 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		Use at an assay dependent concentration. Predicted molecular weight: 23 kDa.
IP		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt,ICC/IF or IHC-P.

Target

Function Rab GTPase key regulator in vesicle trafficking. Essential for maintaining the integrity of the

endosome-trans-Golgi network structure. Together with LRRK2, plays a role in the retrograde trafficking pathway for recycling proteins, such as mannose 6 phosphate receptor (M6PR), between lysosomes and the Golgi apparatus in a retromer-dependent manner. Regulates neuronal process morphology in the intact central nervous system (CNS). May play a role in the formation of typhoid toxin transport intermediates during Salmonella enterica serovar Typhi

(S.Typhi) epithelial cell infection.

Tissue specificity Ubiquitous.

Sequence similaritiesBelongs to the small GTPase superfamily. Rab family.

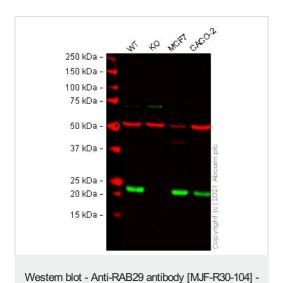
Post-translational In case of Salmonella enterica serovar Typhimurium (S.Typhimurium) infection, is proteolytically modifications cleaved between Gly-41 and Val-42 by the GtgE viral protease encoded on the Gifsy-2 lysogen

bacteriophage, which therefore prevents the recruitment of RAB29 to S.Typhimurium-containing vacuoles. In contrast, no proteolytically cleavage is detected in S.Typhi-infected cells (PubMed:22042847).

Cellular localization

Cell membrane. Cytoplasm. Cytoplasm, perinuclear region. Golgi apparatus. Golgi apparatus, trans-Golgi network. Vacuole. Cytoplasm, cytoskeleton. Colocalizes with LRRK2 along tubular structures emerging from Golgi apparatus (By similarity). Colocalizes with GM130 at the Golgi apparatus. Colocalizes with dynamic tubules emerging from and retracting to the Golgi apparatus. Colocalizes with TGN46 at the trans-Golgi network (TGN). In Salmonella enterica serovar Typhi (S.Typhi) infected epithelial cells, is recruited and colocalized with both S.Typhi-containing vacuoles and dynamic tubules as well as those emerging from the vacuole toward the cell periphery.

Images



BSA and Azide free (ab256548)

All lanes : Anti-RAB29 antibody [MJF-R30-104] (**ab256527**) at 1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: RAB29 knockout A549 cell lysate

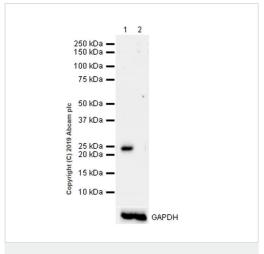
Lane 3 : MCF-7 cell lysate
Lane 4 : Caco-2 cell lysate

Performed under reducing conditions.

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

False colour image of Western blot: Anti-RAB29 antibody [MJF-R30-104] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab256527 was shown to bind specifically to RAB29. A band was observed at 23 kDa in wild-type A549 cell lysates with no signal observed at this size in RAB29 knockout cell line ab280040 (knockout cell lysate None). To generate this image, wild-type and RAB29 knockout A549 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse

lgG H&L (IRDye $^{\tiny{(1)}}$ 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-RAB29 antibody [MJF-R30-104] - BSA and Azide free (ab256548)

All lanes : Anti-RAB29 antibody [MJF-R30-104] (<u>ab256527</u>) at 1/1000 dilution

Lane 1 : Wild type A549 (human lung carcinoma epithelial cell) whole cell lysate

Lane 2: A549 (human lung carcinoma epithelial cell) Rab29 KO whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

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

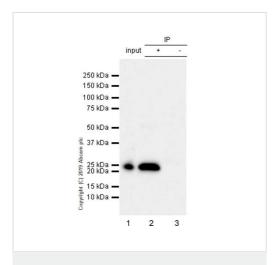
Predicted band size: 23 kDa **Observed band size:** 23 kDa

The lysates were kindly provided by Dr. Dario Alessi, University of Dundee.

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

Exposure Time: 59 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab256527).



Immunoprecipitation - Anti-RAB29 antibody [MJF-R30-104] - BSA and Azide free (ab256548)

RAB29 was immunoprecipitated from 0.35 mg A549 (human lung carcinoma epithelial cell) whole cell lysate with <u>ab256527</u> at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using <u>ab256527</u> at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) was used at 1/5000 dilution.

Lane 1: A549 (human lung carcinoma epithelial cell) whole cell lysate 10ug

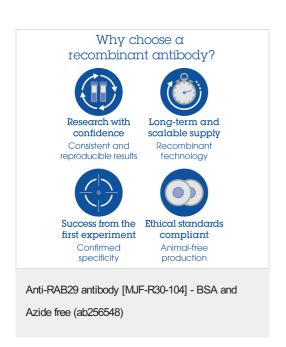
Lane 2: ab256527 IP in A549 whole cell lysate

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of $\underline{ab256527}$ in A549 whole cell lysate

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

Exposure time: 30 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab256527).



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