# abcam

# Product datasheet

# Anti-RAB29 antibody [MJF-R30-124] ab256526





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#### Overview

**Product name** Anti-RAB29 antibody [MJF-R30-124]

Rabbit monoclonal [MJF-R30-124] to RAB29 **Description** 

**Host species** Rabbit

**Tested applications** Suitable for: IP, WB

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

Species reactivity Reacts with: Mouse, Human

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

Positive control WB: Wild type A549, A549, HEK293T cells, HEK293T cells, wildtype C57BL6 mouse lung and

Rab29 knock-out C57BL6 mouse lung lysates. MCF-7, Caco-2 cell lysate. IP: A549 cells.

**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  $\mathsf{RabMAb}^{\mathsf{®}}$  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.



#### **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, 0.05% BSA, 40% Glycerol (glycerin, glycerine)

Purity Protein A purified

Clonality Monoclonal
Clone number MJF-R30-124

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab256526 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
IP		1/30.
WB		1/1000. Predicted molecular weight: 23 kDa.

**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 similarities

Belongs to the small GTPase superfamily. Rab family.

Post-translational modifications

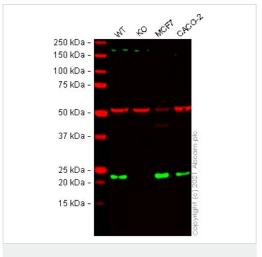
In case of Salmonella enterica serovar Typhimurium (S.Typhimurium) infection, is proteolytically cleaved between Gly-41 and Val-42 by the GtgE viral protease encoded on the Gifsy-2 lysogen bacteriophage, which therefore prevents the recruitment of RAB29 to S.Typhimurium-containing vacuoles. In contrast, 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**



Western blot - Anti-RAB29 antibody [MJF-R30-124] (ab256526)

**All lanes :** Anti-RAB29 antibody [MJF-R30-124] (ab256526) 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-R30-124] 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, ab256526 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 lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-RAB29 antibody [MJF-R30-124] (ab256526)

**All lanes :** Anti-RAB29 antibody [MJF-R30-124] (ab256526) at 1/3000 dilution

Lane 1: wildtype C57BL6 mouse lung tissue lysate

Lane 2: Rab29 knock-out C57BL6 mouse lung tissue lysate

Lysates/proteins at 15 µg per lane.

### Secondary

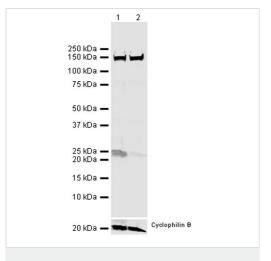
**All lanes :** IR Dye 800CW Goat anti-rabbit lgG (#926-32211) at 1/10000 dilution

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

This antibody reacts with unidentifiable proteins around 30,75 and 150 kDa. This image was kindly provided by Dr. Mark Cookson, NIH.

Blocking/Diluting buffer and concentration: 50% TBST, 50% Odyssey Blocking buffer.

Exposure Time: 3 minutes.



Western blot - Anti-RAB29 antibody [MJF-R30-124] (ab256526)

**All lanes**: Anti-RAB29 antibody [MJF-R30-124] (ab256526) at 1/3000 dilution

**Lane 1 :** HEK293T cells (human embryonic kidney epithelial cell) transiently transfected with non-target siRNA control whole cell lysate

Lane 2: HEK293T cells (human embryonic kidney epithelial cell) transiently transfected with siRNA against Rab29 whole cell lysate

Lysates/proteins at 15 µg per lane.

### **Secondary**

**All lanes :** IR Dye 800CW Goat anti-rabbit lgG (#926-32211) at 1/10000 dilution

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

This antibody reacts with an unidentifiable protein around 150 kDa. This image was kindly provided by Dr. Mark Cookson, NIH.

Blocking/Diluting buffer and concentration: 50% TBST, 50% Odyssey Blocking buffer

Exposure Time: 3 min

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Western blot - Anti-RAB29 antibody [MJF-R30-124] (ab256526)

**All lanes :** Anti-RAB29 antibody [MJF-R30-124] (ab256526) 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

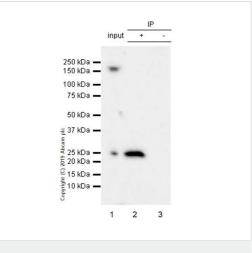
All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051)

**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: 3 min



Immunoprecipitation - Anti-RAB29 antibody [MJF-R30-124] (ab256526)

RAB29 was immunoprecipitated from 0.35 mg A549 (human lung carcinoma epithelial cell) whole cell lysate with ab256526 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab256526 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

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

Lane 2: ab256526 IP in A549 whole cell lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab256526 in A549 whole cell lysate

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

Exposure time: 30 seconds



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