abcam

Product datasheet

Anti-RAB35 antibody ab152138

KO VALIDATED

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Overview

Product name Anti-RAB35 antibody

Description Rabbit polyclonal to RAB35

Host species Rabbit

Tested applications

Suitable for: WB, IHC-P, ICC/IF

Species reactivity

Reacts with: Mouse, Human

Immunogen Recombinant fragment, corresponding to a region within amino acids 1-201 of Human RAB35.

Positive control WB: U-87 MG, SK-N-SH, IMR32, SK-N-AS, HeLa, Jurkat, Caco-2 cell lysate; transfected HEK-

293T cells and mouse brain tissue lysate; IHC-P: Human breast cancer tissue; IF: U87-MG whole

cell.

General notesThe Life Science industry has been in the grips of a reproducibility crisis for a number of years.

Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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 or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.00

Preservative: 0.025% Proclin 300

Constituents: 79% PBS, 20% Glycerol (glycerin, glycerine)

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab152138 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		1/500 - 1/3000. Predicted molecular weight: 23 kDa.
IHC-P		1/100 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol using 10mM Citrate buffer (pH6.0) or Tris-EDTA buffer (pH8.0).
ICC/IF		1/100 - 1/1000.

Target

Function

In the process of endocytosis, essential rate-limiting regulator of a fast recycling pathway back to the plasma membrane. During cytokinesis, required for the postfurrowing terminal steps, namely for intercellular bridge stability and abscission, possibly by controlling phosphatidylinositol 4,5-bis phosphate (PIP2) and SEPT2 localization at the intercellular bridge.

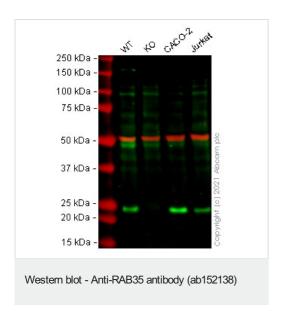
Sequence similarities

Cellular localization

Belongs to the small GTPase superfamily. Rab family.

Cell membrane. Membrane > clathrin-coated pit. Cytoplasmic vesicle > clathrin-coated vesicle. Endosome. Melanosome. Present on sorting endosomes and endosomal tubules. Tends to be enriched in PIP2-positive cell membrane domains. During mitosis, associated with the plasma membrane and present at the ingressing furrow during early cytokinesis as well as at the intercellular bridge later during cytokinesis. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images



All lanes: Anti-RAB35 antibody (ab152138) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2: RAB35 knockout HeLa cell lysate

Lane 3 : Caco-2 cell lysate

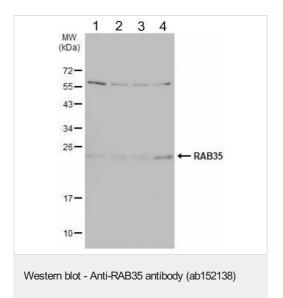
Lane 4 : Jurkat cell lysate

Performed under reducing conditions.

Predicted band size: 23 kDa **Observed band size:** 25 kDa

False colour image of Western blot: Anti-RAB35 antibody staining

at 1/500 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab152138 was shown to bind specifically to RAB35. A band was observed at 25 kDa in wild-type HeLa cell lysates with no signal observed at this size in RAB35 knockout cell line ab265984 (knockout cell lysate ab259076). To generate this image, wild-type and RAB35 knockout HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % 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® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



All lanes: Anti-RAB35 antibody (ab152138) at 1/3000 dilution

Lane 1: U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell lysate

Lane 2 : SK-N-SH (human neuroblastoma cell line) whole cell lysate

Lane 3: IMR32 (human brain neuroblast cell line) whole cell lysate

Lane 4: SK-N-AS (human brain neuroblastoma cell line) whole cell
lysate

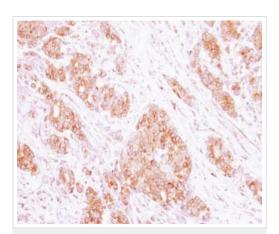
Lysates/proteins at 30 µg per lane.

Secondary

All lanes: HRP-conjugated anti-rabbit lgG antibody

Predicted band size: 23 kDa

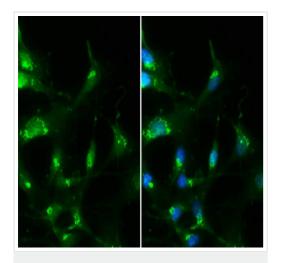
12% SDS-PAGE



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-RAB35 antibody (ab152138)

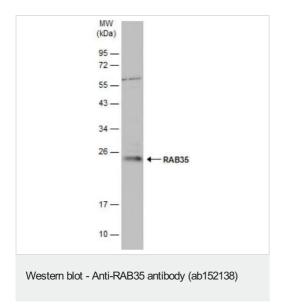
Immunohistochemical analysis of paraffin-embedded human breast cancer tissue staining RAB35 with ab152138 at 1/250 dilution.

Antigen Retrieval: Citrate buffer, pH 6.0, 15 min.



Immunocytochemistry/ Immunofluorescence - Anti-RAB35 antibody (ab152138)

Immunofluorescence analysis of 4% paraformaldehyde-fixed U-87 MG (human glioblastoma-astrocytoma epithelial cell line) whole cell labeling RAB35 with ab152138 at 1/500 dilution. Blue: Hoechst 33342 staining.



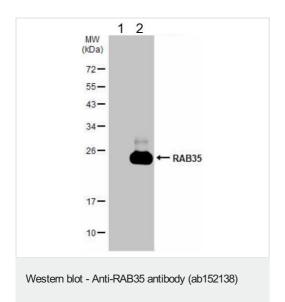
Anti-RAB35 antibody (ab152138) at 1/1000 dilution + Mouse brain tissue lysate at 50 μg

Secondary

HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 23 kDa

12% SDS-PAGE



All lanes: Anti-RAB35 antibody (ab152138) at 1/2000 dilution

Lane 1 : Non-transfected HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 2: Transfected HEK-293T whole cell lysate

Lysates/proteins at 30 µg per lane.

Secondary

All lanes: HRP-conjugated anti-rabbit IgG antibody

Predicted band size: 23 kDa

12% SDS-PAGE

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