

## Product datasheet

# Human RAB8A knockout HeLa cell lysate ab257195

3 Images

### Overview

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<b>Product name</b>	Human RAB8A knockout HeLa cell lysate
<b>Product overview</b>	Knockout cell lysate achieved by CRISPR/Cas9.
<b>Parental Cell Line</b>	HeLa
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, Homozygous: 423 bp deletion in exon 1.
<b>Passage number</b>	<20
<b>Knockout validation</b>	Sanger Sequencing, Western Blot (WB)
<b>Reconstitution notes</b>	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT.

*\*Usage of SDS sample buffer is not recommended with these lyophilized lysates.*

**Notes**

**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found [here](#). Please refer to our lysis protocol for further details on how our lysates are prepared.

**User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage.

Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.

**[See here for more information on knockout cell lysates.](#)**

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It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

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**Tested applications**                      **Suitable for:** WB

## Properties

**Storage instructions** Store at -80°C. Please refer to protocols.

Components	1 kit
ab260140 - Human RAB8A knockout HeLa cell lysate	1 x 100µg
ab255552 - Human wild-type HeLa cell lysate	1 x 100µg

**Cell type** epithelial  
**Disease** Adenocarcinoma  
**Gender** Female  
**STR Analysis** Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10

## Target

**Function** May be involved in vesicular trafficking and neurotransmitter release. Together with RAB11A, RAB3IP, the exocyst complex, PARD3, PRKCI, ANXA2, CDC42 and DNMBP promotes transcytosis of PODXL to the apical membrane initiation sites (AMIS), apical surface formation and lumenogenesis. Together with MYO5B and RAB11A participates in epithelial cell polarization.

**Sequence similarities** Belongs to the small GTPase superfamily. Rab family.

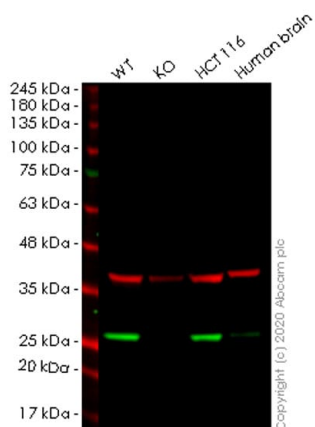
**Cellular localization** Cell membrane. Golgi apparatus. Cytoplasm > perinuclear region. Cell projection. Colocalizes with OPTN at the Golgi complex and in vesicular structures close to the plasma membrane. In the GDP-bound form, present in the perinuclear region. Shows a polarized distribution to distal regions of cell protrusions in the GTP-bound form. Colocalizes with PARD3, PRKCI, EXOC5, OCLN, PODXL and RAB11A in apical membrane initiation sites (AMIS) during the generation of apical surface and lumenogenesis.

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab257195 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: 24 kDa.

## Images



Western blot - Human RAB8A knockout HeLa cell lysate (ab257195)

**Lane 1:** Wild-type HeLa cell lysate (20 µg)

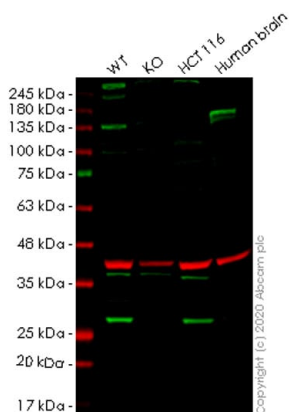
**Lane 2:** RAB8A knockout HeLa cell lysate (20 µg)

**Lane 3:** HCT116 cell lysate (20 µg)

**Lane 4:** Human brain tissue lysate (20 µg)

**Lanes 1-4:** Merged signal (red and green). Green - **ab241061** observed at 24 kDa. Red - loading control, **ab8245** observed at 37 kDa.

**ab241061** Anti-RAB8A antibody [MJF-R22-79-3] was shown to specifically react with RAB8A in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab264993** (knockout cell lysate ab257195) was used. Wild-type and RAB8A knockout samples were subjected to SDS-PAGE. **ab241061** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.



Western blot - Human RAB8A knockout HeLa cell lysate (ab257195)

**Lane 1:** Wild-type HeLa cell lysate (20 µg)

**Lane 2:** RAB8A knockout HeLa cell lysate (20 µg)

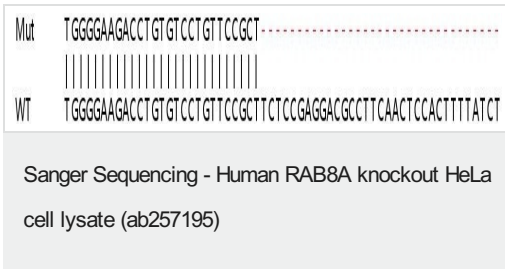
**Lane 3:** HCT116 cell lysate (20 µg)

**Lane 4:** Human brain tissue lysate (20 µg)

**Lanes 1-4:** Merged signal (red and green). Green - **ab188574** observed at 24 kDa. Red - loading control, **ab8245** observed at 37 kDa.

**ab188574** Anti-RAB8A antibody [EPR14873] was shown to specifically react with RAB8A in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab264993** (knockout cell lysate ab257195) was used. Wild-type and RAB8A knockout samples were subjected to SDS-PAGE. **ab188574** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature

before imaging.



Homozygous: 423 bp deletion in exon 1

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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