

Product datasheet

Human RAB9A (Rab9) knockout HeLa cell line  
ab265693

4 Images

Overview

<b>Product name</b>	Human RAB9A (Rab9) knockout HeLa cell line
<b>Parental Cell Line</b>	HeLa
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 3 and Insertion of the selection cassette in exon 3
<b>Passage number</b>	<20
<b>Knockout validation</b>	Sanger Sequencing, Western Blot (WB)
<b>Tested applications</b>	<b>Suitable for:</b> WB
<b>Biosafety level</b>	2
<b>General notes</b>	<p><b>Recommended control:</b> Human wild-type HeLa cell line (<a href="#">ab255928</a>). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.</p> <p><b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p><b>Culture medium:</b> DMEM (High Glucose) + 10% FBS</p> <p><b>Initial handling guidelines:</b> Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability.</p> <ol style="list-style-type: none"> <li>1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.</li> <li>2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.</li> <li>3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of <math>2 \times 10^4</math> cells/cm<sup>2</sup>. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> <p><b>Subculture guidelines:</b></p> <p>All seeding densities should be based on cell counts gained by established methods.</p>

A guide seeding density of  $2 \times 10^4$  cells/cm<sup>2</sup> is recommended.

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.

This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our [limited use license](#) and [patent pages](#).

## Properties

---

<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Viability</b>	~80%
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Cervix
<b>Cell type</b>	epithelial
<b>Disease</b>	Adenocarcinoma
<b>Gender</b>	Female
<b>STR Analysis</b>	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
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

## Target

---

<b>Function</b>	Involved in the transport of proteins between the endosomes and the trans Golgi network.
<b>Sequence similarities</b>	Belongs to the small GTPase superfamily. Rab family.
<b>Cellular localization</b>	Cell membrane. Endoplasmic reticulum membrane. Golgi apparatus membrane.

## Applications

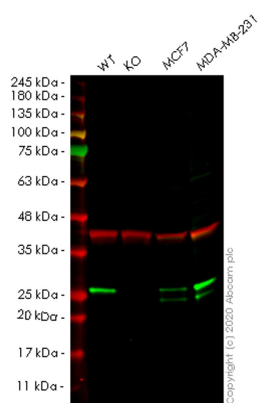
---

**The Abpromise guarantee** Our [Abpromise guarantee](#) covers the use of ab265693 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.

## Images

---



Western blot - Human RAB9A knockout HeLa cell line (ab265693)

**All lanes** : Anti-Rab9 antibody [Mab9] ([ab2810](#)) at 1/1000 dilution

**Lane 1** : Wild-type HeLa cell lysate

**Lane 2** : RAB9A knockout HeLa cell lysate

**Lane 3** : MCF7 cell lysate

**Lane 4** : MDA-MB-231 cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes** : Goat Anti-Rabbit IgG H&L (IRDye® 680RD)

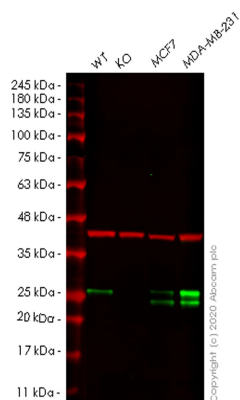
preadsorbed ([ab216777](#)) at 1/10000 dilution

**Predicted band size:** 23 kDa

**Observed band size:** 25 kDa

**Lanes 1-4:** Merged signal (red and green). Green - [ab2810](#) observed at 25 kDa. Red - loading control [ab181602](#) observed at 36 kDa.

[ab2810](#) Anti-Rab9 antibody [Mab9] was shown to specifically react with Rab9 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265693 (knockout cell lysate [ab257625](#)) was used. Wild-type and Rab9 knockout samples were subjected to SDS-PAGE. [ab2810](#) and Anti-GAPDH antibody[EPR16891] - Loading Control ([ab181602](#)) were incubated at room temperature for 2.5 hours 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 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human RAB9A knockout HeLa cell line (ab265693)

**All lanes :** Anti-Rab9 antibody [EPR13272] - Late Endosome Marker ([ab179815](#)) at 1/1000 dilution

**Lane 1 :** Wild-type HeLa cell lysate

**Lane 2 :** RAB9A knockout HeLa cell lysate

**Lane 3 :** MCF7 cell lysate

**Lane 4 :** MDA-MB-231 cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

**Predicted band size:** 23 kDa

**Observed band size:** 25 kDa

**Lanes 1-4:** Merged signal (red and green). Green - [ab179815](#) observed at 25 kDa. Red - loading control [ab8245](#) observed at 36 kDa.

[ab179815](#) Anti-Rab9 antibody [EPR13272] was shown to specifically react with Rab9 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265693 (knockout cell lysate [ab257625](#)) was used. Wild-type and Rab9 knockout samples were subjected to SDS-PAGE. [ab179815](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated at room temperature for 2.5 hours 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 20000 dilution for 1 hour at room temperature before imaging.

Mut	TCATCACTTTTTAAAGTAATTCTCCTTGGAGATGGTGGAGTTGGGAAGAGTTCACCTTAT
WT	TCATCACTTTTTAAAGTAATTCTCCTTGGAGATGGTGGAGTTGGGAAGAGTTCACCTTAT

Sanger Sequencing - Human RAB9A knockout HeLa cell line (ab265693)

Allele-1: 1 bp insertion in exon 3.

Mut	TAACAATGGCAGGAAATCA*****Insertion*****TCACCTTTTAAAGTAATTCT
WT	TAACAATGGCAGGAAATCA TCACCTTTTAAAGTAATTCT

Sanger Sequencing - Human RAB9A knockout HeLa cell line (ab265693)

Allele-2: Insertion of the selection cassette in exon 3.

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

### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

### Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors