

Product datasheet

Human RANBP1 knockout HEK293T cell line ab266202

3 Images

Overview

<b>Product name</b>	Human RANBP1 knockout HEK293T cell line
<b>Parental Cell Line</b>	HEK293T
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in exon 2
<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 HEK293T cell line (<a href="#">ab255449</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 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 <b>culture medium</b>, wash vial with an additional 0.8 ml <b>culture medium</b> (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 <b>culture medium</b> and count using a haemocytometer (<a href="#">Click here to view haemocytometer protocol</a>) or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 2x10<sup>4</sup> cells/cm<sup>2</sup>. This should allow for confluency within 48 hours. 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. A guide seeding density of 2x10<sup>4</sup> cells/cm<sup>2</sup> is recommended for confluency (80-90% confluence)</p>

within 48 hours.

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.

[Click here to view the Mammalian cell tissue culture protocol](#)

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## Properties

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<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Viability</b>	~90%
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Kidney
<b>Cell type</b>	epithelial
<b>STR Analysis</b>	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether
<b>Purity</b>	Immunogen affinity purified

## Target

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<b>Function</b>	Inhibits GTP exchange on Ran. Forms a Ran-GTP-RANBP1 trimeric complex. Increase GTP hydrolysis induced by the Ran GTPase activating protein RANGAP1. May act in an intracellular signaling pathway which may control the progression through the cell cycle by regulating the transport of protein and nucleic acids across the nuclear membrane.
<b>Sequence similarities</b>	Belongs to the RANBP1 family. Contains 1 RanBD1 domain.

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## Applications

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Our [Abpromise guarantee](#) covers the use of **ab266202** in the following tested applications.

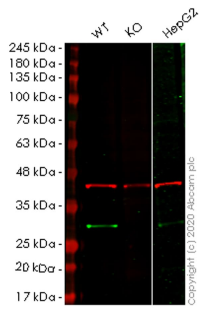
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 23 kDa.

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## Images

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Western blot - Human RANBP1 knockout HEK293T cell line (ab266202)

**All lanes** : Anti-RanBP1 antibody [EPR6796(B)] (ab133550) at 1/1000 dilution

**Lane 1** : Wild-type HEK293T cell lysate

**Lane 2** : RANBP1 knockout HEK293T cell lysate

**Lane 3** : HepG2 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:** 30 kDa

[why is the actual band size different from the predicted?](#)

**Lanes 1-4:** Merged signal (red and green). Green - ab133550 observed at 30 kDa. Red - loading control ab8245 observed at 36 kDa.

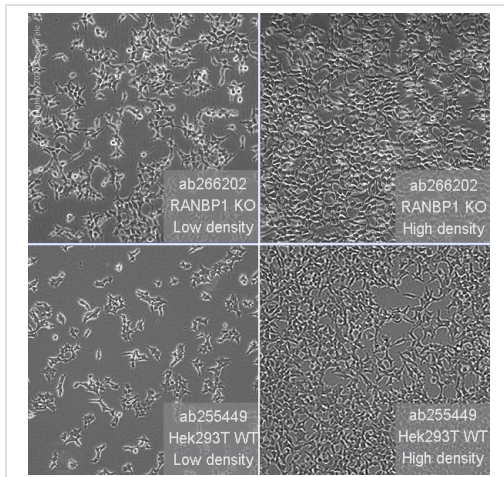
ab133550 Anti-RanBP1 antibody [EPR6796(B)] was shown to specifically react with RanBP1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266202 (knockout cell lysate ab258168) was used. Wild-type and RanBP1 knockout samples were subjected to SDS-PAGE. ab133550 and Anti-GAPDH 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.

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Mut  GTTGGACTCGTCTGTATTCT*****Insertion*****CAGT GGAAGTATCATGGTCC
      |||
WT   GTTGGACTCGTCTGTATTCT                      CAGT GGAAGTATCATGGTCC
  
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Sanger Sequencing - Human RANBP1 knockout HEK293T cell line (ab266202)

Homozygous: Insertion of the selection cassette in exon 2



Representative images of RANBP1 knockout HEK293T cells, low and high confluency examples (top left and right respectively) and wild-type HEK293T cells, low and high confluency (bottom left and right respectively) showing typical adherent, epithelial-like morphology. Images were captured at 10X magnification using an EVOS M5000 microscope.

Cell Culture - Human RANBP1 knockout HEK293T cell line (ab266202)

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