

# Human ADAR (ADAR1) knockout HEK-293T cell line ab266846

4 Images

### Overview

<b>Product name</b>	Human ADAR (ADAR1) knockout HEK-293T cell line
<b>Parental Cell Line</b>	HEK293T
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, 17 bp deletion in exon 2 and 1 bp insertion 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 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. A guide seeding density of <math>2 \times 10^4</math> cells/cm<sup>2</sup> is recommended.</p>

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.

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We will provide viable cells that proliferate on revival.

## Properties

<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<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>Antibiotic resistance</b>	Puromycin 1.00µg/ml
<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

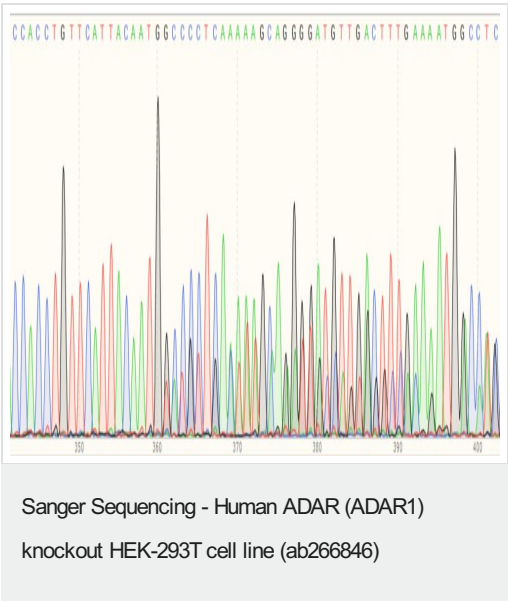
<b>Function</b>	Converts multiple adenosines to inosines and creates I/U mismatched base pairs in double-helical RNA substrates without apparent sequence specificity. Has been found to modify more frequently adenosines in AU-rich regions, probably due to the relative ease of melting A/U base pairs as compared to G/C pairs. Functions to modify viral RNA genomes and may be responsible for hypermutation of certain negative-stranded viruses. Edits the messenger RNAs for glutamate receptor (GLUR) subunits by site-selective adenosine deamination. Produces low-level editing at the GLUR-B Q/R site, but edits efficiently at the R/G site and HOTSPOT1. Binds to short interfering RNAs (siRNA) without editing them and suppresses siRNA-mediated RNA interference. Binds to ILF3/NF90 and up-regulates ILF3-mediated gene expression.
<b>Tissue specificity</b>	Ubiquitously expressed, highest levels were found in brain and lung.
<b>Involvement in disease</b>	Defects in ADAR are a cause of dyschromatosis symmetrical hereditaria (DSH) [MIM:127400]; also known as reticulate acropigmentation of Dohi. DSH is a pigmentary genodermatosis of autosomal dominant inheritance characterized by a mixture of hyperpigmented and hypopigmented macules distributed on the dorsal parts of the hands and feet.
<b>Sequence similarities</b>	Contains 1 A to I editase domain. Contains 2 DRADA repeats. Contains 3 DRBM (double-stranded RNA-binding) domains.
<b>Post-translational modifications</b>	Sumoylation reduces RNA-editing activity.
<b>Cellular localization</b>	Cytoplasm. Nucleus > nucleolus. Isoform 1 is found predominantly in cytoplasm but appears to shuttle between the cytoplasm and nucleus. Isoform 5 is found exclusively in the nucleolus.

Applications

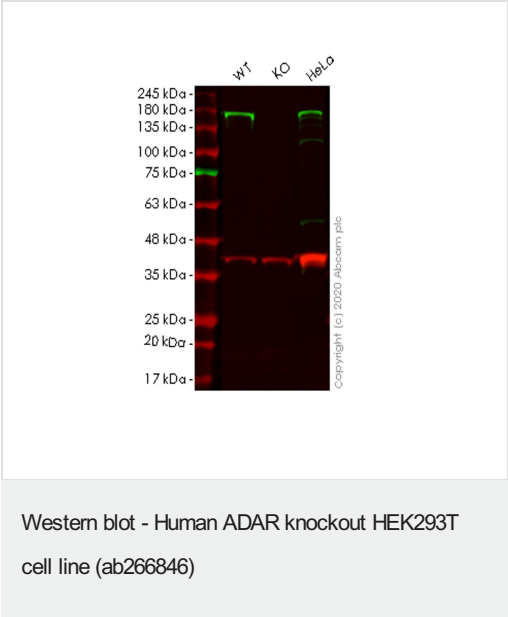
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab266846 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 136 kDa.

Images



Sequencing chromatogram displaying sequence edit in exon 2



**All lanes** : Anti-ADAR1 antibody [EPR7033] (**ab126745**) at 1/1000 dilution

- Lane 1** : Wild-type HEK293T cell lysate
- Lane 2** : ADAR knockout HEK293T cell lysate
- Lane 3** : HeLa 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:** 136 kDa  
**Observed band size:** 130 kDa

**Lanes 1-3:** Merged signal (red and green). Green - **ab126745** observed at 130 kDa. Red - loading control **ab8245** observed at 36 kDa.

**ab126745** Anti-ADAR1 antibody [EPR7033] was shown to specifically react with ADAR1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266846 (knockout cell lysate **ab257131**) was used. Wild-type and ADAR1 knockout samples were subjected to SDS-PAGE. **ab126745** 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 20000 dilution for 1 hour at room temperature before imaging.

Mut	CCACCTGTTCAATGAGCCCTCAAAA-.....TGAAATGCCAG
WT	CCACCTGTTCAATGAGCCCTCAAAAGCAGGATGTTGACTTTGAAATGCCAG

Sanger Sequencing - Human ADAR knockout  
HEK293T cell line (ab266846)

Allele-1: 17 bp deletion in exon2

Mut	CCACCTGTTCAATGAGCCCTCAAAAAGCAGGATGTTGACTTTGAAATGCCA
WT	CCACCTGTTCAATGAGCCCTCAAAA GCAGGATGTTGACTTTGAAATGCCA

Sanger Sequencing - Human ADAR knockout  
HEK293T cell line (ab266846)

Allele-2: 1 bp insertion in exon 2.

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

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