

Human EIF2AK4 (GCN2) knockout HEK-293T cell line ab267246

4 Images

Overview

Product name	Human EIF2AK4 (GCN2) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 16 bp deletion in exon 9 and 1 bp insertion in exon 9
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Recommended control: Human wild-type HEK293T cell line (ab255449). 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>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>Initial handling guidelines: 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"> 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 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. 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 2×10^4 cells/cm². Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² 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

Number of cells	1 x 10 ⁶ cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
STR Analysis	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
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

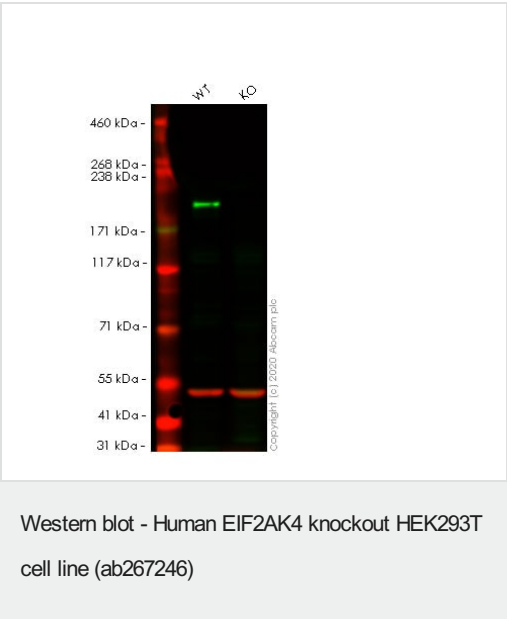
Function	Can phosphorylate the alpha subunit of EIF2 and may mediate translational control.
Tissue specificity	Widely expressed.
Sequence similarities	Belongs to the protein kinase superfamily. Ser/Thr protein kinase family. GCN2 subfamily. Contains 2 protein kinase domains. Contains 1 RWD domain.
Domain	Kinase domain 1 is a degenerate kinase domain. RWD domain is reported to interact with GCN1L1.
Post-translational modifications	Autophosphorylated on threonine residues.

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab267246 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: 187 kDa.



All lanes : Anti-GCN2 antibody [EPR5970(2)] ([ab134053](#)) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate

Lane 2 : EIF2AK4 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

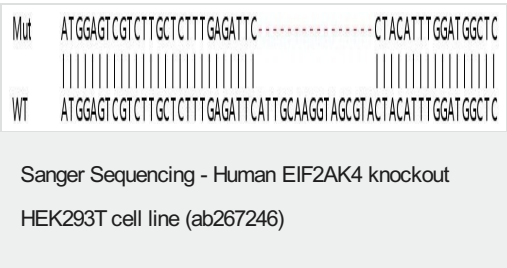
Performed under reducing conditions.

Predicted band size: 187 kDa

Observed band size: 187 kDa

Lanes 1- 2: Merged signal (red and green). Green - [ab134053](#) observed at 187 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control ([ab7291](#)) observed at 50 kDa.

[ab134053](#) was shown to react with GCN2 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line ab267246 (knockout cell lysate [ab256902](#)) was used. Wild-type HEK-293T and EIF2AK4 knockout HEK-293T cell lysates were subjected to SDS-PAGE. [ab134053](#) and Anti-alpha Tubulin antibody [DM1A] - Loading Control ([ab7291](#)) overnight at 4°C at a 1 in 1000 Dilution and a 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.



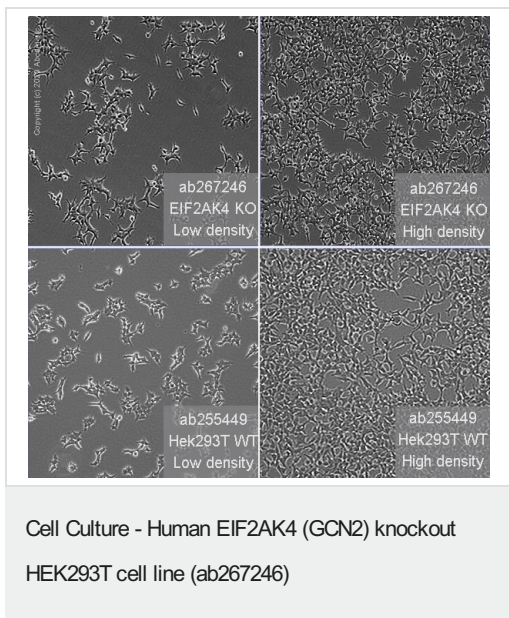
Allele-1: 16 bp deletion in exon9

Mut	ATGGAGTCGTCCTTGCTCTTTGAGATTCATTGCAAGGTAGCGTACTACATTGGATGGCT
WT	ATGGAGTCGTCCTTGCTCTTTGAGATTCATTGCAAGGTAGCGTACTACATTGGATGGCT

Sanger Sequencing - Human EIF2AK4 knockout

HEK293T cell line (ab267246)

Allele-2: 1 bp insertion in exon 9.



Representative images of EIF2AK4 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 a EVOS XL Core microscope.

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