abcam

Product datasheet

Human APBB1 (FE65) knockout HEK-293T cell line ab267294

3 Images

Overview

Product name	Human APBB1 (FE65) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 4 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **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. 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 $2x10^4$ cells/cm^2. 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% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 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 and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

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
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	Transcription coregulator that can have both coactivator and corepressor functions. Adapter protein that forms a transcriptionally active complex with the gamma-secretase-derived amyloid precursor protein (APP) intracellular domain. Plays a central role in the response to DNA damage by translocating to the nucleus and inducing apoptosis. May act by specifically recognizing and binding histone H2AX phosphorylated on 'Tyr-142' (H2AXY142ph) at double-strand breaks (DSBs), recruiting other pro-apoptosis factors such as MAPK8/JNK1. Required for histone H4 acetylation at double-strand breaks (DSBs). Its ability to specifically bind modified histones and chromatin modifying enzymes such as KAT5/TIP60, probably explains its trancription activation activity. Function in association with TSHZ3, SET and HDAC factors as a transcriptional repressor, that inhibits the expression of CASP4. Associates with chromatin in a region surrounding the CASP4 transcriptional start site(s).
Tissue specificity	Highly expressed in brain; strongly reduced in post-mortem elderly subjects with Alzheimer disease.
Sequence similarities	Contains 2 PID domains. Contains 1 WW domain.
Post-translational modifications	Phosphorylated following nuclear translocation. Phosphorylation at Tyr-546 enhances the transcription activation activity and reduces the affinity with RASD1/DEXRAS1.
Cellular localization	Cell membrane. Cytoplasm. Nucleus. Cell projection > growth cone. Colocalizes with TSHZ3 in axonal growth cone (By similarity). In normal conditions, it mainly localizes to the cytoplasm, while a small fraction is tethered to the cell membrane via its interaction with APP. Following exposure to DNA damaging agents, it is released from cell membrane and translocates to the nucleus. Nuclear translocation is under the regulation of APP. Colocalizes with TSHZ3 in the nucleus.

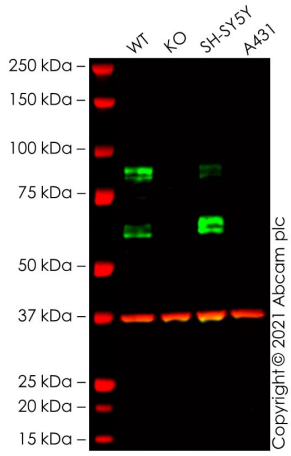
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab267294 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.

Images



Western blot - Human APBB1 (FE65) knockout HEK-293T cell line (ab267294)

All lanes : Anti-FE65 antibody [EPR3538] (**ab91650**) at 1/2000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : APBB1 knockout HEK-293T cell lysate
Lane 3 : SH-SY5Y cell lysate
Lane 4 : A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

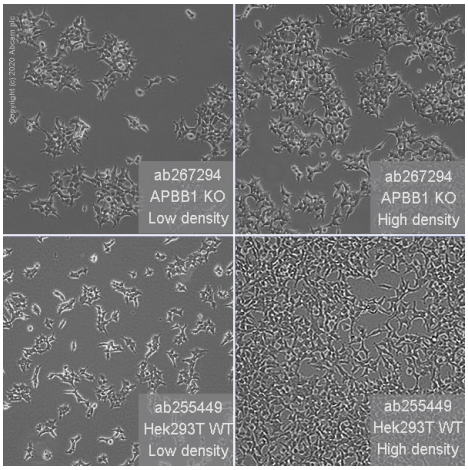
Observed band size: 65 kDa

False colour image of Western blot: Anti-FE65 antibody [EPR3538] staining at 1/2000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab91650** was shown to bind specifically to FE65. A band was observed at 65/85 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in APBB1 knockout cell line ab267294 (knockout cell lysate **ab257833**). To generate this image, wild-type and APBB1 knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % milk in TBS-0.1% Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD)

preabsorbed (**ab216776**) at 1/20000 dilution.

Sanger Sequencing - Human APBB1 knockout HEK293T cell line (ab267294)

Homozygous: 4 bp deletion in exon2



Cell Culture - Human APBB1 (FE65) knockout HEK293T cell line (ab267294)

Representative images of APBB1 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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