abcam

Product datasheet

Human PSENEN (PEN2) knockout HEK-293 cell line ab273720

3 Images

Overview

Product name Human PSENEN (PEN2) knockout HEK-293 cell line

Parental Cell Line HEK-293
Organism Human

Mutation description Knockout achieved by CRISPR/Cas9; X = 1 bp deletion, 1 bp insertion; Frameshift: 100%

Passage number <20

Knockout validation Next Generation Sequencing (NGS), Western Blot (WB)

Tested applications Suitable for: WB, Next Generation Sequencing

Biosafety level 2

General notesRecommended control: Human wild-type HEK-293 cell line (<u>ab259776</u>). 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.

 $\textbf{Cryopreservation cell medium:} \ \ \textbf{Cell Freezing Medium-DMSO Serum free media, contains}$

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 2x10⁴ 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.

Subculture guidelines:

All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm² is recommended.

1

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

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

Mycoplasma free

1 x 10⁶ cells/vial, 1 mL **Number of cells**

Adherent/Suspension Adherent **Tissue** Kidney Cell type epithelial Gender Female

Yes Storage instructions Shipped on Dry Ice. Store in liquid nitrogen.

Storage buffer Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	Essential subunit of the gamma-secretase complex, an endoprotease complex that catalyzes the	
	intramembrane cleavage of integral membrane proteins such as Notch receptors and APP (beta-	
	amyloid precursor protein). Probably represents the last step of maturation of gamma-secretase,	
	facilitating endoproteolysis of presenilin and conferring gamma-secretase activity.	
Tissue specificity	Widely expressed. Expressed in leukocytes, lung, placenta, small intestine, liver, kidney, spleen thymus, skeletal muscle, heart and brain.	
Sequence similarities	Belongs to the PEN-2 family.	
Cellular localization	Endoplasmic reticulum membrane. Golgi apparatus > Golgi stack membrane. Predominantly	
	located in the endoplasmic reticulum and in the cis-Golgi.	

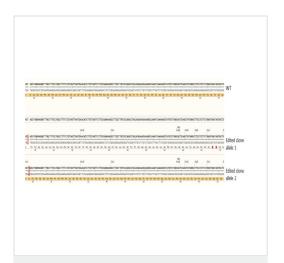
Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab273720 in the following tested applications.

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

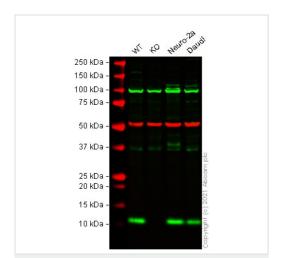
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 12 kDa.
Next Generation Sequencing		Use at an assay dependent concentration.

Images



Next Generation Sequencing - Human PSENEN (PEN2) knockout HEK-293 cell line (ab273720)

1 bp deletion (allele 1) and 1 bp insertion (allele 2) after Tyr18 of the WT protein



Western blot - Human PSENEN (PEN2) knockout HEK-293 cell line (ab273720)

All lanes : Anti-PEN2 antibody [EPR9200] (<u>ab154830</u>) at 1/1000 dilution

Lane 1: Wild-type HEK-293 cell lysate

Lane 2: PSENEN knockout HEK-293 cell lysate

Lane 3: Neuro-2a cell lysate

Lane 4: Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 12 kDa

Observed band size: 12 kDa

False colour image of Western blot: Anti-PEN2 antibody
[EPR9200] staining at 1/1000 dilution, shown in green; Mouse antiAlpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000
dilution, shown in red. In Western blot, ab154830 was shown to bind
specifically to PEN2. A band was observed at 12 kDa in wild-type
HEK-293 cell lysates with no signal observed at this size in
PSENEN knockout cell line ab273720 (knockout cell lysate
ab273772). To generate this image, wild-type and PSENEN

knockout HEK-293 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % 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.

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(PEN2) knockout HEK-293 cell line (ab273720)

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