abcam

Product datasheet

Human SMARCD1 knockout HEK-293T cell line ab266458

4 Images

Overview

Product name Human SMARCD1 knockout HEK-293T cell line

Parental Cell Line HEK293T
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 1 and 2 bp insertion in exon 1

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Tested applications Suitable for: WB

Biosafety level 2

General notesRecommended 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.

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

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 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 10⁶ 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

Involved in chromatin remodeling. Belongs to the neural progenitors-specific chromatin remodeling complex (npBAF complex) and the neuron-specific chromatin remodeling complex (nBAF complex). During neural development a switch from a stem/progenitor to a post-mitotic chromatin remodeling mechanism occurs as neurons exit the cell cycle and become committed to their adult state. The transition from proliferating neural stem/progenitor cells to post-mitotic neurons requires a switch in subunit composition of the npBAF and nBAF complexes. As neural progenitors exit mitosis and differentiate into neurons, npBAF complexes which contain ACTL6A/BAF53A and PHF10/BAF45A, are exchanged for homologous alternative ACTL6B/BAF53B and DPF1/BAF45B or DPF3/BAF45C subunits in neuron-specific complexes (nBAF). The npBAF complex is essential for the self-renewal/proliferative capacity of the multipotent neural stem cells. The nBAF complex along with CREST plays a role regulating the activity of genes essential for dendrite growth (By similarity). Has a strong influence on the Vitamin D-mediated transcriptional activity from an enhancer Vitamin D receptor element (VDRE). May be a link between mammalian SWI-SNF-like chromatin remodeling complexes and the vitamin D receptor (VDR) heterodimer. Mediates critical interactions between nuclear receptors and the BRG1/SMARCA4 chromatin-remodeling complex for transactivation. Also involved in vitamin Dcoupled transcription regulation via its association with the WINAC complex, a chromatinremodeling complex recruited by vitamin D receptor (VDR), which is required for the ligand-bound VDR-mediated transrepression of the CYP27B1 gene.

Tissue specificity

Expressed in all tissues tested, including brain, heart, kidney, liver, lung, muscle, pancreas and

placenta

Sequence similarities

Belongs to the SMARCD family. Contains 1 SWIB domain.

Applications

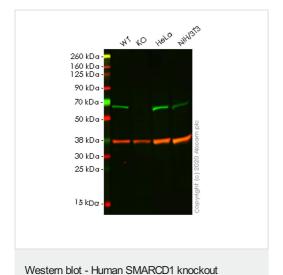
The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab266458 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: 58 kDa.

Images



HEK293T cell line (ab266458)

All lanes : Anti-SMARCD1 antibody [EPR23170-71] (ab245222) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T (Human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 2 : SMARCD1 knockout HEK-293T (Human epithelial cell

line from embryonic kidney transformed with large T antigen) whole cell lysate

Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 4: NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 58 kDa

Observed band size: 58 kDa

Lanes 1-4: Merged signal (red and green). Green - <u>ab245222</u> observed at 58 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

<u>ab245222</u> Anti-SMARCD1 antibody [EPR23170-71] was shown to specifically react with SMARCD1 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab266458

(knockout cell lysate ab259143) was used. Wild-type and SMARCD1 knockout samples were subjected to SDS-PAGE. ab245222 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.



Sanger Sequencing - Human SMARCD1 knockout HEK293T cell line (ab266458)

Allele-1: 2 bp insertion in exon1



Sanger Sequencing - Human SMARCD1 knockout HEK293T cell line (ab266458)

Human SMARCD1 knockout HEK293T cell line (ab266458)

Allele-2: 1 bp deletion in exon 1.

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