abcam

Product datasheet

Human SMARCC1 (BAF155) knockout HEK-293 cell line ab261854

3 Images

Overview

Product name Human SMARCC1 (BAF155) knockout HEK-293 cell line

Parental Cell Line HEK-293
Organism Human

Mutation description Knockout achieved by CRISPR/Cas9; X = 1 bp insertion, 2 bp insertion, 1 bp insertion

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.

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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
Gender Female
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 transcriptional activation and repression of select genes by chromatin remodeling (alteration of DNA-nucleosome topology). May stimulate the ATPase activity of the catalytic subunit of the complex. Also involved in vitamin D-coupled transcription regulation via its association with the WINAC complex, a chromatin-remodeling complex recruited by vitamin D receptor (VDR), which is required for the ligand-bound VDR-mediated transrepression of the CYP27B1 gene. 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.

Tissue specificity

Expressed in brain, heart, muscle, placenta, lung, liver, muscle, kidney and pancreas.

Sequence similarities

Belongs to the SMARCC family.
Contains 1 SANT domain.
Contains 1 SWIRM domain.

Post-translational modifications

Phosphorylated on undefined residues at the G2/M transition by ERK1 and other kinases. This may contribute to cell cycle specific inactivation of remodeling complexes containing the phosphorylated protein.

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab261854 in the following tested applications.

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

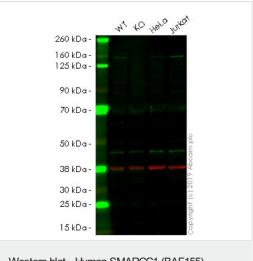
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 123 kDa.
Next Generation Sequencing		Use at an assay dependent concentration.

Images



Next Generation Sequencing - Human SMARCC1 (BAF155) knockout HEK-293 cell line (ab261854)

2 bp insertion (allele 1), 1 bp insertion and 1 bp deletion (allele 2), and 1 bp insertion (allele 3) after Asp174 of the WT protein



Western blot - Human SMARCC1 (BAF155) knockout HEK-293 cell line (ab261854)

All lanes: Anti-SMARCC1/BAF155 antibody [EPR12389] (ab172636) at 1/5000 dilution

Lane 1 : Wild-type HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2: SMARCC1 knockout HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

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

Lane 4: Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

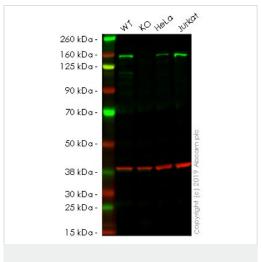
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 123 kDa Observed band size: 123 kDa

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab172636</u> observed at 123 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

<u>ab172636</u> was shown to specifically react with SMARCC1 in wild-type HEK-293 cells as signal was lost in SMARCC1 knockout cell line ab261854 (knockout cell lysate <u>ab261662</u>). Wild-type and SMARCC1 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab172636 and <u>ab8245</u> (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human SMARCC1 (BAF155) knockout HEK-293 cell line (ab261854)

All lanes : Anti-SMARCC1/BAF155 antibody [EPR12395] - ChIP Grade (ab172638) at 1/5000 dilution

Lane 1 : Wild-type HEK293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2: SMARCC1 knockout HEK293 (Human epithelial cell line from embryonic kidney) whole cell lysate

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

Lane 4 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 123 kDa

Lanes 1 - 4: Merged signal (red and green). Green - <u>ab172638</u> observed at 123 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

<u>ab172638</u> was shown to recognize in wild-type HEK-293 cells as signal was lost at the expected MW in SMARCC1 knockout cell line ab261854 (knockout cell lysate <u>ab261662</u>). Additional cross-reactive bands were observed in the wild-type and knockout cell lysate. Wild-type and SMARCC1 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% milk. Ab172638 and <u>ab8245</u> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/5000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

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