abcam

Product datasheet

Human SMARCA4 (BRG1) knockout HEK-293T cell lysate ab263853

5 Images

Overview

Product name	Human SMARCA4 (BRG1) knockout HEK-293T cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 4 and 7 bp deletion in exon 4.
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
Notes	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. 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**.
Tested applications	**Suitable for:** WB

Properties

Storage instructions Store at -80°C. Please refer to protocols.

Components	1 kit
ab255536 - Human SMARCA4 knockout HEK293T cell lysate	1 x 100µg
ab255553 - Human wild-type HEK293T cell lysate	1 x 100µg

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

Target

Function Transcriptional coactivator cooperating with nuclear hormone receptors to potentiate transcriptional activation. Component of the CREST-BRG1 complex, a multiprotein complex that regulates promoter activation by orchestrating a calcium-dependent release of a repressor complex and a recruitment of an activator complex. In resting neurons, transcription of the c-FOS promoter is inhibited by BRG1-dependent recruitment of a phospho-RB1-HDAC repressor complex. Upon calcium influx, RB1 is dephosphorylated by calcineurin, which leads to release of the repressor complex. At the same time, there is increased recruitment of CREBBP to the promoter by a CREST-dependent mechanism, which leads to transcriptional activation. The CREST-BRG1 complex also binds to the NR2B promoter, and activity-dependent induction of NR2B expression involves a release of HDAC1 and recruitment of CREBBP. 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. SMARCA4/BAF190A may promote neural stem cell self-renewal/proliferation by enhancing Notch-dependent proliferative signals, while concurrently making the neural stem cell insensitive to SHH-dependent differentiating cues (By similarity). 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. Acts as a corepressor of ZEB1 to regulate E-cadherin transcription and is required for induction of epithelial-mesenchymal transition (EMT) by ZEB1.

Tissue specificity Colocalizes with ZEB1 in E-cadherin-negative cells from established lines, and stroma of normal colon as well as in de-differentiated epithelial cells at the invasion front of colorectal carcinomas (at protein level).

Involvement in disease	Defects in SMARCA4 are the cause of rhabdoid tumor predisposition syndrome type 2 (RTPS2) [MIM:613325]. RTPS2 is a familial cancer syndrome predisposing to renal or extrarenal malignant rhabdoid tumors and to a variety of tumors of the central nervous system, including choroid plexus carcinoma, medulloblastoma, and central primitive neuroectodermal tumors. Rhabdoid tumors are the most aggressive and lethal malignancies occurring in early childhood.
Sequence similarities	Belongs to the SNF2/RAD54 helicase family. Contains 1 bromo domain. Contains 1 helicase ATP-binding domain. Contains 1 helicase C-terminal domain. Contains 1 HSA domain.
Post-translational modifications	Phosphorylated upon DNA damage, probably by ATM or ATR.
Cellular localization	Nucleus.

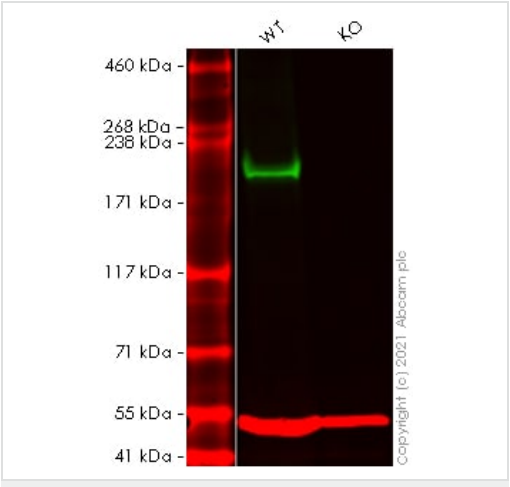
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab263853 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.

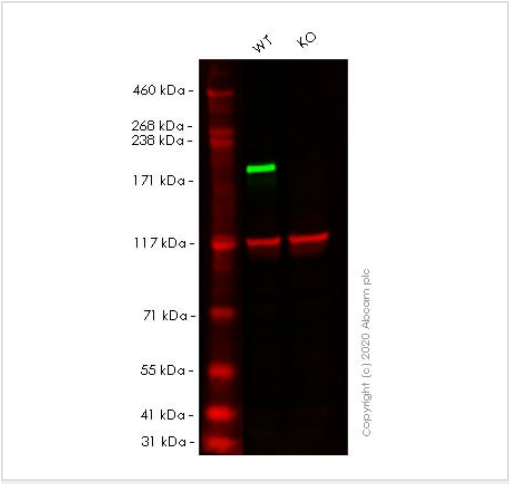
Images



Western blot - Human SMARCA4 (BRG1) knockout HEK-293T cell lysate (ab263853)

Lane 1: Wild-type HEK-293T cell lysate 20 µg
Lane 2: SMARCA4 knockout HEK-293T cell lysate 20 µg
False colour image of Western blot: Anti-BRG1 antibody [EPNCIR111A] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab110641** was shown to bind specifically to BRG1. A band was observed at 185 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in SMARCA4 knockout cell line **ab255432** (knockout cell lysate ab263853). To generate this image, wild-type and SMARCA4 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 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.

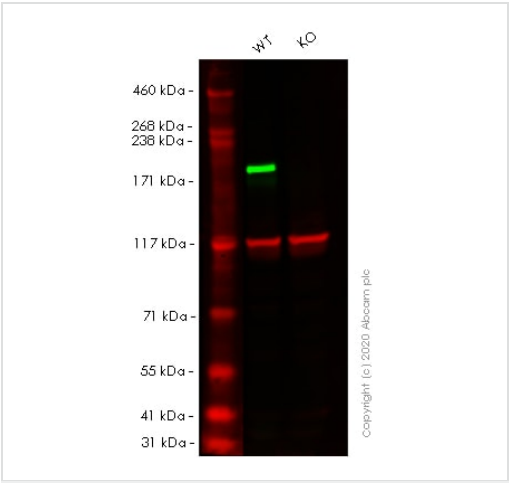


Western blot - Human SMARCA4 knockout HEK293T cell lysate (ab263853)

Lane 1: Wild-type HEK-293T cell lysate (20 ug)
Lane 2: SMARCA4 knockout HEK-293T cell lysate (20 ug)

Lanes 1- 2: Merged signal (red and green). Green - **ab110641** observed at 185 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab110641 was shown to react with SMARCA4 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab255432** (knockout cell lysate ab263853) was used. Wild-type HEK-293T and SMARCA4 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab110641** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 10000 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.



Western blot - Human SMARCA4 (BRG1) knockout HEK293T cell lysate (ab263853)

Lane 1: Wild-type HEK-293T cell lysate (20µg)

Lane 2: SMARCA4 knockout HEK-293T cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab108318** observed at 185 kDa. Red - loading control **ab130007** observed at 124 kDa.

ab108318 Recombinant Anti-BRG1 antibody [EPR3912] was shown to specifically react with SMARCA4 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab255432** (knockout cell lysate ab263853) was used. Wild-type and SMARCA4 knockout samples were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab108318** and Anti-Vinculin antibody [VIN-54] were incubated overnight at 4°C at 1 in 10000 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 SMARCA4 knockout HEK293T cell lysate (ab263853)

Allele-1: 7 bp deletion in exon 4

Sanger Sequencing - Human SMARCA4 knockout HEK293T cell lysate (ab263853)

Allele-2: 1 bp deletion in exon 4

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