abcam

Product datasheet

Human KDM5C (Jarid1C / SMCX) knockout HEK-293T cell line ab266252

3 Images

Overview

Product name	Human KDM5C (Jarid1C / SMCX) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 17 bp deletion in exon 4 and 5 bp deletion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
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.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Viability	~80%
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	Histone demethylase that specifically demethylates 'Lys-4' of histone H3, thereby playing a central role in histone code. Does not demethylate histone H3 'Lys-9', H3 'Lys-27', H3 'Lys-36', H3 'Lys-79' or H4 'Lys-20'. Demethylates trimethylated and dimethylated but not monomethylated H3 'Lys-4'. Participates in transcriptional repression of neuronal genes by recruiting histone deacetylases and REST at neuron-restrictive silencer elements.
Tissue specificity	Expressed in all tissues examined. Highest levels found in brain and skeletal muscle.
Involvement in disease	Defects in KDM5C are the cause of mental retardation syndromic X-linked JARID1C-related (MRXSJ) [MIM:300534]. MRXSJ is characterized by significantly sub-average general intellectual functioning associated with impairments in adaptative behavior and manifested during the developmental period. MRXSJ patients manifest mental retardation associated with variable features such as slowly progressive spastic paraplegia, seizures, facial dysmorphism.
Sequence similarities	Belongs to the JARID1 histone demethylase family. Contains 1 ARID domain. Contains 1 JmjC domain. Contains 1 JmjN domain. Contains 2 PHD-type zinc fingers.
Domain	The first PHD-type zinc finger domain recognizes and binds H3-K9Me3. Both the JmjC domain and the JmjN domain are required for enzymatic activity.
Cellular localization	Nucleus.

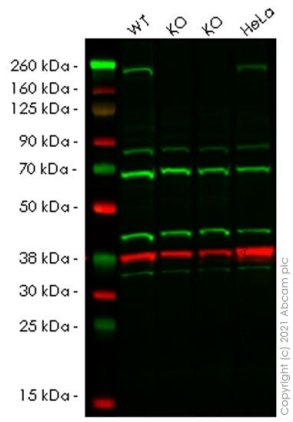
Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab266252 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: 175 kDa.

Images



Western blot - Human KDM5C (Jarid1C / SMCX) knockout HEK-293T cell line (ab266252)

All lanes : Anti-KDM5C / Jarid1C / SMCX antibody [EPR23932-18] (ab259913) at 1/1000 dilution

Lane 1 : Wild-type HEK293T (human embryonic kidney epithelial cell), whole cell lysate
Lane 2 : Human KDM5C (Jarid1C / SMCX) knockout HEK-293T cell line (ab266252)
Lane 3 : Human KDM5C (Jarid1C / SMCX) knockout HEK-293T cell line (ab266251)
Lane 4 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (IRDye® 800CW) (ab216773) and Goat Anti-Mouse IgG H&L (IRDye® 680RD) (ab216776)

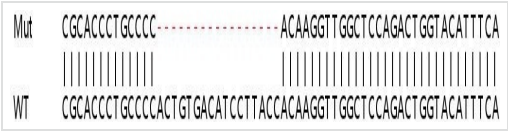
Predicted band size: 175 kDa
Observed band size: 180 kDa

Blocking and Diluting buffer and concentration: 5% NFDM/TBST

Lanes 1-4: Merged signal (red and green). Green - ab259913 observed at 180 kDa. Red - loading control ab8245 (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

ab259913 Anti-KDM5C / Jarid1C / SMCX antibody [EPR23932-18] was shown to specifically react with KDM5C / Jarid1C / SMCX in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266251 (knockout cell lysate ab257494) and ab266252 (knockout cell lysate ab257495) were used. Wild-type and KDM5C / Jarid1C / SMCX knockout samples were subjected

to SDS-PAGE. ab259913 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at 4? overnight 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 10000 dilution for 1 hour at room temperature before imaging.



Sanger Sequencing - Human KDM5C knockout HEK293T cell line (ab266252)

Allele-1: 17 bp deletion in exon4

Sanger Sequencing - Human KDM5C knockout HEK293T cell line (ab266252)

Allele-2: 5 bp deletion in exon 4.

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