## abcam

## Product datasheet

## Human KDM1A (LSDl) knockout HeLa cell line ab265790

3 Images

## Overview

Product name
Parental Cell Line
Organism
Mutation description
Passage number
Knockout validation
Tested applications
Biosafety level
General notes

Human KDM1A (LSD1) knockout HeLa cell line
HeLa
Human
Knockout achieved by using CRISPR/Cas9, Homozygous: 4 bp deletion in exon 1 <20

Sanger Sequencing, Western Blot (WB)
Suitable for: WB

## 2

Recommended control: Human wild-type HeLa cell line (ab255928). 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 $-80^{\circ} \mathrm{C}$. Storage at $-80^{\circ} \mathrm{C}$ may result in loss of viability.

1. Thaw the vial in $37^{\circ} \mathrm{C}$ water bath for approximately $1-2$ minutes.
2. Transfer the cell suspension ( 0.8 mL ) to a $15 \mathrm{~mL} / 50 \mathrm{~mL}$ 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 $201 \mathrm{xg}(\mathrm{rcf})$ 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 $2 \times 10^{4}$ cells $/ \mathrm{cm}^{2}$. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.
4. Incubate the culture at $37^{\circ} \mathrm{C}$ incubator with $5 \% \mathrm{CO}_{2}$. 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 $2 \times 10^{4}$ cells $/ \mathrm{cm}^{2}$ 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.

We will provide viable cells that proliferate on revival.

## Properties

| Number of cells | $1 \times 10^{6}$ cells/vial, 1 mL |
| :--- | :--- |
| Adherent /Suspension | Adherent |
| Tissue | Cervix |
| Cell type | epithelial |
| Disease | Adenocarcinoma |
| Gender | Female |
| STR Analysis | Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 |
|  | TH01:7 TPOX: 8,12 CSF1PO: 9, 10 |
| Antibiotic resistance | Puromycin $1.00 \mu \mathrm{~g} / \mathrm{ml}$ |
| 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 demethylates both 'Lys-4' (H3K4me) and 'Lys-9' (H3K9me) of histone H 3 , thereby acting as a coactivator or a corepressor, depending on the context. Acts by oxidizing the substrate by FAD to generate the corresponding imine that is subsequently hydrolyzed. Acts as a corepressor by mediating demethylation of H 3 K 4 me , a specific tag for epigenetic transcriptional activation. Demethylates both mono- (H3K4me1) and di-methylated (H3K4me2) H3K4me. May play a role in the repression of neuronal genes. Alone, it is unable to demethylate H3K4me on nucleosomes and requires the presence of RCOR1/CoREST to achieve such activity. Also acts as a coactivator of androgen receptor (ANDR)-dependent transcription, by being recruited to ANDR target genes and mediating demethylation of H 3 K 9 me , a specific tag for epigenetic transcriptional repression. The presence of PRKCB in ANDR-containing complexes, which mediates phosphorylation of 'Thr-6' of histone H3 (H3T6ph), a specific tag that prevents demethylation H3K4me, prevents H3K4me demethylase activity of KDM1A. Demethylates dimethylated 'Lys-370' of p53/TP53 which prevents interaction of p53/TP53 with TP53BP1 and represses p53/TP53-mediated transcriptional activation. Demethylates and stabilizes the DNA methylase DNMT1. Required for gastrulation during embryogenesis. |
| Tissue specificity | Ubiquitously expressed. |
| Sequence similarities | Belongs to the flavin monoamine oxidase family. Contains 1 SWIRM domain. |
| Domain | The SWIRM domain may act as an anchor site for a histone tail. |

## Applications

The Abpromise guarantee
Our Abpromise guarantee covers the use of ab265790 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 <br> weight: 92 kDa. |

Images


Western blot - Human KDM1A (LSD1) knockout HeLa cell line (ab265790)

Anti-KDM1/LSD1 antibody [1B2F2] (ab69535) + KDM1 knockout HeLa cell lysate at $20 \mu \mathrm{~g}$

Performed under reducing conditions.

Predicted band size: 92 kDa
Observed band size: 110 kDa

Lanes 1-2: Merged signal (red and green). Green - ab69535 observed at 110 kDa. Red - Anti-GAPDH antibody[EPR16891] Loading Control (ab181602) observed at 37 kDa .
ab69535 was shown to react with KDM1/LSD1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265790 (knockout cell lysate ab256965) was used. Wildtype HeLa and KDM1 knockout HeLa 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. ab69535 and Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) overnight at $4^{\circ} \mathrm{C}$ at $1 \mathrm{\mu g} / \mathrm{ml}$ and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse lgG H\&L (IRDye ${ }^{\circledR} 800 \mathrm{CW}$ ) preadsorbed (ab216772) and Goat Anti-Rabbit lgG H\&L (IRDye ${ }^{\circledR} 680$ RD) preadsorbed (ab216777) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.


All lanes : Anti-KDM1/LSD1 antibody [EPR6825] - Nuclear Marker and ChIP Grade (ab129195) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : KDM1A knockout HeLa cell lysate

Lysates/proteins at $20 \mu \mathrm{~g}$ per lane.

Performed under reducing conditions.

Predicted band size: 92 kDa
Observed band size: 110 kDa

Lanes 1-2: Merged signal (red and green). Green - ab129195 observed at 110 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa .
ab129195 was shown to react with KDM1/LSD1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265790 (knockout cell lysate ab256965) was used. Wildtype HeLa and KDM1 knockout HeLa 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. ab129195 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at $4^{\circ} \mathrm{C}$ at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H\&L (IRDye ${ }^{\circledR 8} 800 \mathrm{CW}$ ) preadsorbed (ab216773) and Goat anti-Mouse IgG H\&L (IRDye ${ }^{\circledR} 680$ RD) preadsorbed ( $\mathbf{( a b 2 1 6 7 7 6}$ ) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

Homozygous: 4 bp deletion in exon 1.


Sanger Sequencing - Human KDM1A knockout
HeLa cell line (ab265790)

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