

Product datasheet

Human KDM1B (LSD2 / AOF1) knockout HeLa cell lysate ab258016

3 Images

Overview

Product name	Human KDM1B (LSD2 / AOF1) knockout HeLa cell lysate
Product overview	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 2 bp deletion in exon3 and 5 bp deletion in exon3.
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. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

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.](#)

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Tested applications **Suitable for:** WB

Properties

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

Components	1 kit
ab260432 - Human KDM1B knockout HeLa cell lysate	1 x 100µg
ab255929 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial
Disease Adenocarcinoma
Gender Female
STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 WWA: 16, 18 TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

Target

Function Histone demethylase that demethylates 'Lys-4' of histone H3, a specific tag for epigenetic transcriptional activation, thereby acting as a corepressor. Required for de novo DNA methylation of a subset of imprinted genes during oogenesis. Acts by oxidizing the substrate by FAD to generate the corresponding imine that is subsequently hydrolyzed. Demethylates both mono- and di-methylated 'Lys-4' of histone H3. Has no effect on tri-methylated 'Lys-4', mono-, di- or tri-methylated 'Lys-9', mono-, di- or tri-methylated 'Lys-27', mono-, di- or tri-methylated 'Lys-36' of histone H3, or on mono-, di- or tri-methylated 'Lys-20' of histone H4.

Sequence similarities Belongs to the flavin monoamine oxidase family.
Contains 1 CW-type zinc finger.
Contains 1 SWIRM domain.

Domain The SWIRM domain may act as an anchor site for a histone tail.

Cellular localization Nucleus.

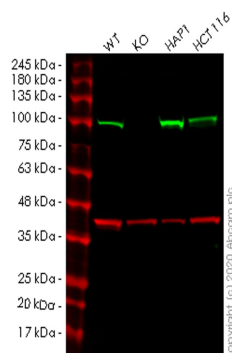
Form There are 3 isoforms produced by alternative splicing.

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab258016 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: 92 kDa.

Images



Western blot - Human KDM1B knockdown HeLa cell lysate (ab258016)

Lane 1: Wild-type HeLa cell lysate (20 ug)

Lane 2: KDM1B knockout HeLa cell lysate (20 ug)

Lane 3: HAP1 cell lysate (20 ug)

Lane 4: HCT116 cell lysate (20 ug)

ab193080 was shown to specifically react with LSD2 / AOF1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab265969** (knockout cell lysate ab258016) was used. Wild-type and LSD2 / AOF1 knockout samples were subjected to SDS-PAGE. **ab193080** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2.5 hours 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.

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Mut  TTGCAGATTATTTAATGTAATGGCAACTCC-----GGAGGACAAAGAAAAAGCATCTTT
      |||
WT   TTGCAGATTATTTAATGTAATGGCAACTCCACGGGGGAGGACAAAGAAAAAGCATCTTT
  
```

Sanger Sequencing - Human KDM1B knockout HeLa cell lysate (ab258016)

Allele-1: 5 bp deletion in exon3

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Mut  TTGCAGATTATTTAATGTAATGGCAACTCC--GGGGGAGGACAAAGAAAAAGCATCTTT
      |||
WT   TTGCAGATTATTTAATGTAATGGCAACTCCACGGGGGAGGACAAAGAAAAAGCATCTTT
  
```

Sanger Sequencing - Human KDM1B knockout HeLa cell lysate (ab258016)

Allele-2: 2 bp deletion in exon3

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