abcam

## Product datasheet

# Human JMJD6 knockout HEK-293T cell lysate ab257490

3 Images

### Overview

| | |
|---|---|
| **Product name** | Human JMJD6 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, Homozygous: 1 bp deletion in exon 1. |
| **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.<br>**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.<br><br>**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.<br><br>Access thousands of knockout cell lysates, generated from commonly used cancer cell lines.<br>**See here for more information on knockout cell lysates.**<br><br>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.<br>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.<br><br>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 |
|---|---|
| ab260996 - Human JMJD6 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** Dioxygenase that can both act as a histone arginine demethylase and a lysyl-hydroxylase. Acts as a lysyl-hydroxylase that catalyzes 5-hydroxylation on specific lysine residues of target proteins such as U2AF2/U2AF65 and LUC7L2. Acts as a regulator of RNA splicing by mediating 5-hydroxylation of U2AF2/U2AF65, affecting the pre-mRNA splicing activity of U2AF2/U2AF65. In addition to peptidyl-lysine 5-dioxygenase activity, may act as a RNA hydroxylase, as suggested by its ability to bind single strand RNA. Also acts as an arginine demethylase which demethylates histone H3 at 'Arg-2' (H3R2me) and histone H4 at 'Arg-3' (H4R3me), thereby playing a role in histone code. However, histone arginine demethylation may not constitute the primary activity in vivo. Has no histone lysine demethylase activity. Required for differentiation of multiple organs during embryogenesis. Acts as a key regulator of hematopoietic differentiation: required for angiogenic sprouting by regulating the pre-mRNA splicing activity of U2AF2/U2AF65. Seems to be necessary for the regulation of macrophage cytokine responses.

**Tissue specificity** Highly expressed in the heart, skeletal muscle and kidney. Expressed at moderate or low level in brain, placenta, lung, liver, pancreas, spleen, thymus, prostate, testis and ovary. Up-regulated in many patients with chronic pancreatitis. Expressed in nursing thymic epithelial cells.

**Sequence similarities** Belongs to the JMJD6 family.
Contains 1 JmjC domain.

**Domain** The nuclear localization signal motifs are necessary and sufficient to target it into the nucleus.

**Cellular localization** Nucleus > nucleoplasm. Nucleus > nucleolus. Mainly found throughout the nucleoplasm outside of regions containing heterochromatic DNA, with some localization in nucleolus. During mitosis, excluded from the nucleus and reappears in the telophase of the cell cycle.

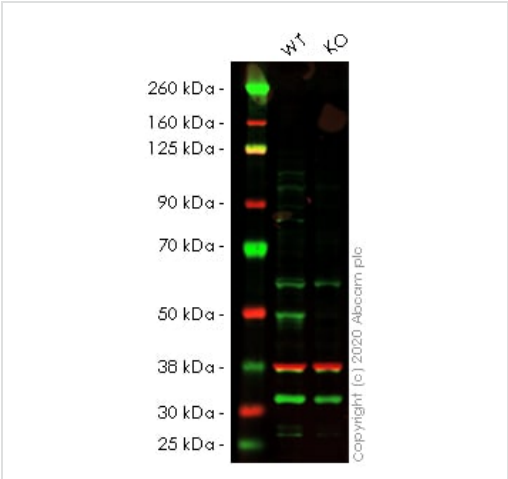
## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab257490 in the following tested applications.

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

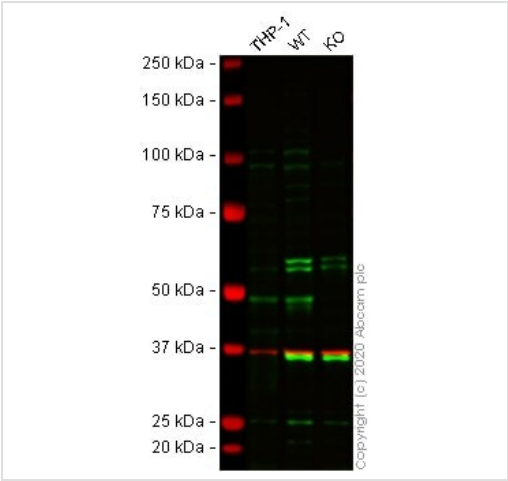
| Application | Abreviews | Notes |
|---|---|---|
| **WB** | | Use at an assay dependent concentration. Predicted molecular weight: 46 kDa. |

## Images




Western blot - Human JMJD6 knockout HEK293T cell lysate (ab257490)

**Lane 1:** Wild-type HEK293T cell lysate 20 ug
**Lane 2:** JMJD6 knockout HEK293T cell lysate 20 ug
**Lanes 1 - 2:**Merged signal (red and green). Green - **ab64575** observed at 50 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.
**ab64575** was shown to react with JMJD6 in wild-type HEK293T cells in Western blot with loss of signal observed in JMJD6 knockout cell line **ab266402** (JMJD6 knockout cell lysate ab257490). Wild-type and JMJD6 knockout HEK293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab64575** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.




Western blot - Human JMJD6 knockout HEK293T cell lysate

**Lane 1:** THP-1 cell lysate (20 ug)

**Lane 2:** Wild-type HEK293T cell lysate (20 ug)

**Lane 3:** JMJD6 knockout HEK293T cell lysate (20 ug)

**Lanes 1 - 3:** Merged signal (red and green). Green - **ab64575** observed at 50 kDa. Red - loading control, **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

**ab64575** was shown to react with JMJD6 in wild-type HEK-293 cells in western blot. Loss of signal was observed when JMJD6 knockout cell lysate (ab257490) was used. Wild-type and JMJD6 knockout HEK-293 cell lysates were subjected to SDS-PAGE. Membranes were blocked in non-mammalian (TBS-based) blocking solution before incubation with **ab64575** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.


Sanger Sequencing - Human JMJD6 knockout HEK293T cell lysate (ab257490)

Homozygous: 1 bp deletion in exon 1

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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