abcam

Product datasheet

Anti-KDM6A / UTX antibody ab36938

KO VALIDATED

★★★★★ ★ Abreviews <u>33 References</u> 5 Images

Overview

Product name	Anti-KDM6A / UTX antibody
Description	Rabbit polyclonal to KDM6A / UTX
Host species	Rabbit
Specificity	Replenishment batches of our polyclonal antibody, ab36938 are tested in WB. Previous batches were additionally validated in ChIP and ICC/IF. These applications are still expected to work and are covered by our Abpromise guarantee. You may also be interested in our alternative recombinant antibody, <u>ab253183</u> .
Tested applications	Suitable for: ChIP, WB, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
	Predicted to work with: Xenopus laevis 🛛 🔺
Immunogen	Synthetic peptide corresponding to Human KDM6A/ UTX aa 400-500 conjugated to keyhole limpet haemocyanin. (Peptide available as <u>ab36937</u>)
Positive control	WB: HeLa and HAP1 cell lysates; Whole NIH3T3 cell lysate (no shRNA and Control shRNA) ICC: MCF7 cells.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.
	If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or - 80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40

Constituent: PBSBatches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising
agent. If you would like information about the formulation of a specific lot, please contact our
scientific support team who will be happy to help.PurityImmunogen affinity purifiedClonalityPolyclonalIsotypeIgG

Preservative: 0.02% Sodium azide

Applications

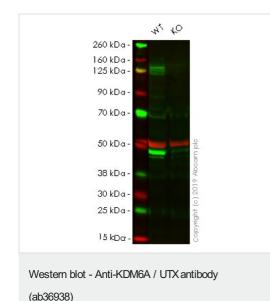
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab36938 in the following tested applications.

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

Application	Abreviews	Notes
ChIP		Use at an assay dependent concentration. PubMed: 26306033
WB	★ ★ ★ ☆ ☆ ☆ <u>(3)</u>	Use a concentration of 1 $\mu\text{g/ml}.$ Predicted molecular weight: 154 kDa.
ICC/IF		Use a concentration of 1 µg/ml.

Histone demethylase that specifically demethylates 'Lys-27' of histone H3, thereby playing a central role in histone code. Demethylates trimethylated and dimethylated but not monomethylated H3 'Lys-27'. Plays a central role in regulation of posterior development, by regulating HOX gene expression. Demethylation of 'Lys-27' of histone H3 is concomitent with methylation of 'Lys-4' of
histone H3, and regulates the recruitment of the PRC1 complex and monoubiquitination of histone H2A.
Belongs to the UTX family. Contains 1 JmjC domain. Contains 8 TPR repeats.
Nucleus.

Images



All lanes : Anti-KDM6A / UTX antibody (ab36938) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate Lane 2 : KDM6A knockout HeLa cell lysate

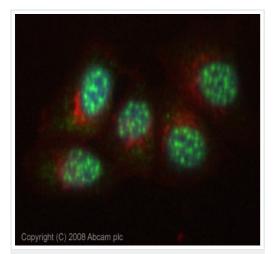
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

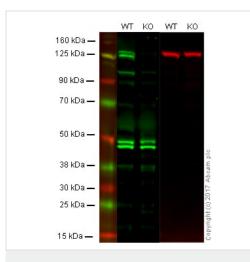
Predicted band size: 154 kDa Observed band size: 154 -157 kDa

Lanes 1-2: Merged signal (red and green). Green - ab36938 observed at 154 -157 kDa. Red - Anti-GAPDH antibody [6C5] -Loading Control (<u>ab8245</u>) observed at 37 kDa.

ab36938 was shown to react with KDM6A / UTX in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab265110** (knockout cell lysate **ab257214**) was used. Wild-type HeLa and KDM6A 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. ab36938 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®]680RD) 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.



Immunocytochemistry/ Immunofluorescence - Anti-KDM6A / UTX antibody (ab36938) ICC/IF image of ab36938 stained MCF7 cells. The cells were 4% PFA fixed (10 min), permabilised in 0.1% PBS-Tween (20 min) and incubated with the antibody (ab36938, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue). This antibody also gave a positive IF result in HeLa cells fixed in 4% PFA at 1ug/ml and HeLa and MCF7 100% methanol fixed cells at 1ug/ml. However, this Fast-Track antibody is not yet fully characterised. This image represents inconclusive preliminary data.



Western blot - Anti-KDM6A / UTX antibody (ab36938)

Lanes 1-2 : Anti-KDM6A / UTX antibody (ab36938) at 0.7 µg/ml Lanes 3-4 : Anti-Vinculin antibody [SPM227] (<u>ab18058</u>) at 1/20000 dilution

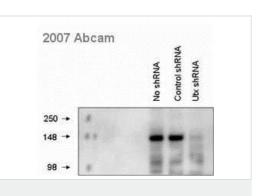
Lanes 1 & 3 : Wild-type HAP1 whole cell lysate Lanes 2 & 4 : KDM6A knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 154 kDa

Lanes 1 - 2: Green - ab36938 observed at 154 kDa.

Lanes 3 - 4: Red - loading control, <u>ab18058</u>, observed at 130 kDa. ab36938 was shown to recognize KDM6A in wild-type HAP1 cells as signal was lost at the expected MW in KDM6A knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and KDM6A knockout samples were subjected to SDS-PAGE. Ab36938 and <u>ab18058</u> (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 0.7 µg/ml and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preabsorbed



Western blot - Anti-KDM6A / UTX antibody (ab36938) This image is courtesy of an anonymous Abreview <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

All lanes : Anti-KDM6A / UTX antibody (ab36938) at 1 µg/ml

Lane 1 : Whole NIH3T3 cell lysate (no shRNA) Lane 2 : Whole NIH3T3 cell lysate (Control shRNA) Lane 3 : Whole NIH3T3 cell lysate (Utx shRNA)

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : HRP-conjugated Goat anti-Rabbit polyclonal

Developed using the ECL technique.

Performed under reducing conditions.

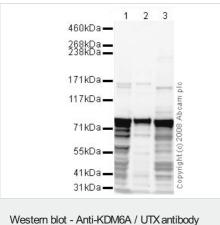
Predicted band size: 154 kDa Observed band size: 148 kDa

Exposure time: 10 seconds

Lane 3 is NIH3T3 cells transduced with lentivirus expressing shRNA for mouse Utx.

5% milk was used as the blocking agent (incubated for 1 hour at 25°C).

ab36938 was diluted with TBS+0.1% Tween 20 and incubated for 1 hour at 25°C.



(ab36938)

All lanes : Anti-KDM6A / UTX antibody (ab36938) at 1 µg/ml

Lane 1 : Testis (Rat) Tissue Lysate - normal tissue (<u>ab29388</u>) Lane 2 : Testis (Mouse) Tissue Lysate Lane 3 : Liver (Rat) Tissue Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Rabbit lgG -H&L- Pre Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 154 kDaObserved band size: 154,157 kDaAdditional bands at: 80 kDa. We are unsure as to the identity of these extra bands.

This Fast-Track antibody is not yet fully characterised. This image represents inconclusive preliminary data.

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