

Product datasheet

Anti-KMT6 / EZH2 antibody - ChIP Grade ab3748

★★★★☆ 20 Abreviews 55 References 10 Images

Overview

Product name	Anti-KMT6 / EZH2 antibody - ChIP Grade
Description	Rabbit polyclonal to KMT6 / EZH2 - ChIP Grade
Host species	Rabbit
Specificity	ab3748 antibody recognises EZH2 in cells transfected with HA-EZH2 in IP.
Tested applications	Suitable for: IHC-FoFr, WB, IP, ChIP, IHC-P, ICC/IF, RIP
Species reactivity	Reacts with: Mouse, Rat, Cow, Human, Xenopus laevis Predicted to work with: Zebrafish 
Immunogen	Synthetic peptide conjugated to KLH derived from within residues 50 - 150 of Human EZH2. Read Abcam's proprietary immunogen policy (Peptide available as ab13744 .)
Positive control	WB: Hek293 whole cell lysate; EZH2 recombinant ICC: mouse embryonic stem cells IP: U2OS cells transfected with 4ug of Myc-EZH2
General notes	The Drosophila protein Enhancer of Zeste is a member of the Polycomb group, which maintains homeotic gene repression and is thought to control gene expression by regulating chromatin. EZH2 is thought to perform a similar role.

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	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
Purity	Immunogen affinity purified
Primary antibody notes	The Drosophila protein Enhancer of Zeste is a member of the Polycomb group, which maintains homeotic gene repression and is thought to control gene expression by regulating chromatin. EZH2 is thought to perform a similar role.
Clonality	Polyclonal
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab3748** in the following tested applications.

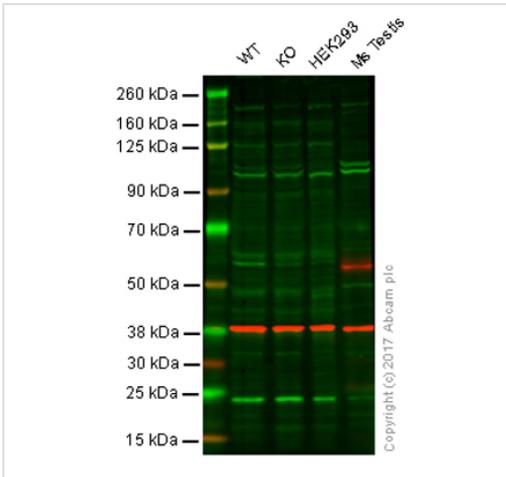
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-FoFr		Use at an assay dependent concentration.
WB	★★★★☆	Use a concentration of 0.4 - 1 µg/ml. Can be blocked with KMT6 / EZH2 peptide (ab13744) .
IP	★★★★☆	Use at an assay dependent concentration.
ChIP	★★★★☆	Use at an assay dependent concentration.
IHC-P	★★★★★	Use at an assay dependent concentration.
ICC/IF	★★★★☆	1/100.
RIP		Use at an assay dependent concentration. PubMed: 19571010

Target

Function	Polycomb group (PcG) protein. Catalytic subunit of the PRC2/EED-EZH2 complex, which methylates 'Lys-9' and 'Lys-27' of histone H3, leading to transcriptional repression of the affected target gene. Able to mono-, di- and trimethylate 'Lys-27' of histone H3 to form H3K27me1, H3K27me2 and H3K27me3, respectively. Compared to EZH2-containing complexes, it is more abundant in embryonic stem cells and plays a major role in forming H3K27me3, which is required for embryonic stem cell identity and proper differentiation. The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two epigenetic repression systems. Genes repressed by the PRC2/EED-EZH2 complex include HOXC8, HOXA9, MYT1, CDKN2A and retinoic acid target genes.
Tissue specificity	Expressed in many tissues. Overexpressed in numerous tumor types including carcinomas of the breast, colon, larynx, lymphoma and testis.
Sequence similarities	Belongs to the histone-lysine methyltransferase family. EZ subfamily. Contains 1 SET domain.
Developmental stage	Expression decreases during senescence of embryonic fibroblasts (HEFs). Expression peaks at the G1/S phase boundary.
Post-translational modifications	Phosphorylated by AKT1. Phosphorylation by AKT1 reduces methyltransferase activity.
Cellular localization	Nucleus.

Images



Western blot - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

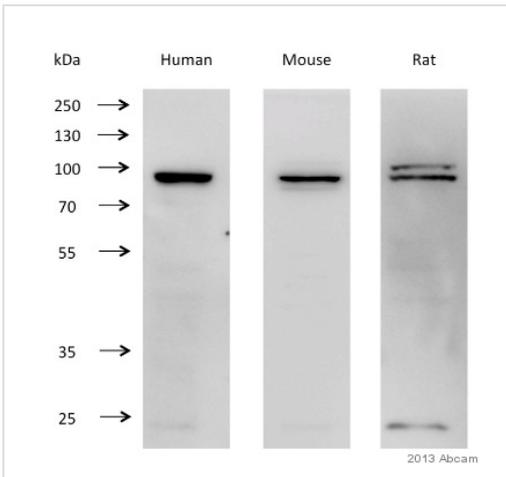
Lane 2: EZH2 p65 knockout HAP1 cell lysate (20 µg)

Lane 3: HEK293 cell lysate (20 µg)

Lane 4: Mouse testis tissue lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green – ab3748 observed at 92 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab3748 was shown not to specifically recognize KMT6/EZH2 when EZH2 knockout samples were used. Wild-type and EZH2 knockout samples were subjected to SDS-PAGE. ab3748 and ab8245 (loading control to GAPDH) were diluted to 1 µg/ml and 1/1000 respectively and incubated overnight at 4°C. 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 1/10 000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

This image is courtesy of an anonymous Abreview

All lanes : Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748) at 1/1000 dilution

Lane 1 : HEK293 cell lysate

Lane 2 : Mouse hippocampus cell lysate

Lane 3 : Rat primary hippocampal neurons cell lysate

Lysates/proteins at 30 µg per lane.

Secondary

All lanes : HRP-conjugated Mouse anti-rabbit IgG at 1/5000 dilution

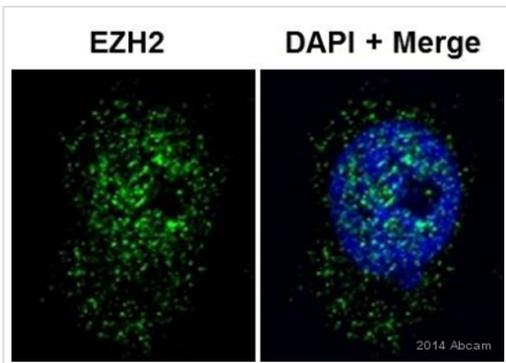
Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 85 kDa [why is the actual band size different from the predicted?](#)

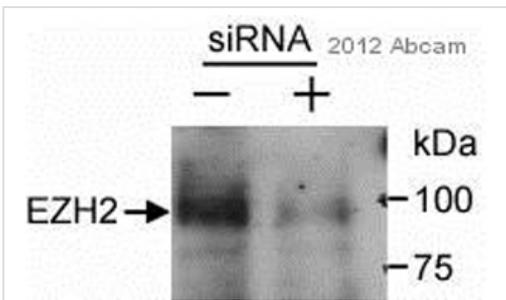
Additional bands at: 100 kDa (possible non-specific binding), 20 kDa (possible non-specific binding)

Exposure time: 20 seconds



Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)
Image courtesy of an anonymous Abreview.

ab3748 staining KMT6/EZH2 in the HeLa cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde permeabilized with 0.2% Triton X-100 in PBS and blocked with 2% BSA for 45 minutes at RT. Samples were incubated with primary antibody (1/2550 in PBS + 2% BSA) for 14 hours at 4°C. An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody (1:10000).



Western blot - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)
Image courtesy of an anonymous Abreview.

All lanes : Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748) at 1/1000 dilution

Lane 1 : Human glioma cell LN229

Lane 2 : Human glioma cell LN229 transfected with a Ezh2-siRNA

Lysates/proteins at 20 µg per lane.

Developed using the ECL technique.

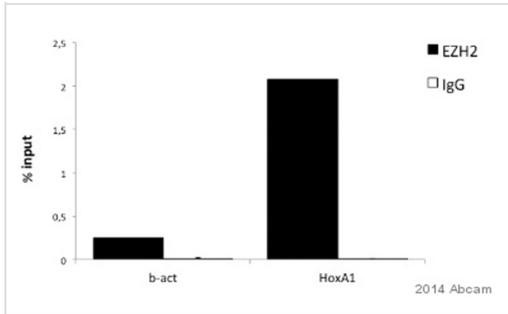
Performed under reducing conditions.

Additional bands at: 90 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 1 minute

Western blot analysis of Human glioma cell lysate (20µg/lane) labelling KMT6/EMT2 with ab3748 at 1/1000 for 16 hours at 4°C. A goat anti-rabbit HRP(1/10000) was used as the secondary antibody.

Blocking was with 5% milk for 1 hour at 25°C.



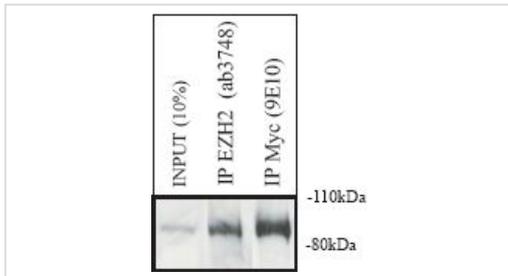
ChIP - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

This image is courtesy of an anonymous Abreview

ChIP analysis using ab3748 binding KMT6/EZH2 in rat Hippocampal primary Neurons. Cells were cross-linked for 10 minutes with 1% formaldehyde. Samples were incubated with primary antibody (1:100) for 16 hours at 4°C. Protein binding was detected using real-time PCR.

Positive control: HoxA1, a PolyComb repressed gene in Hippocampus.

Negative Control: b-act, an active gene, and normal Rabbit IgG.

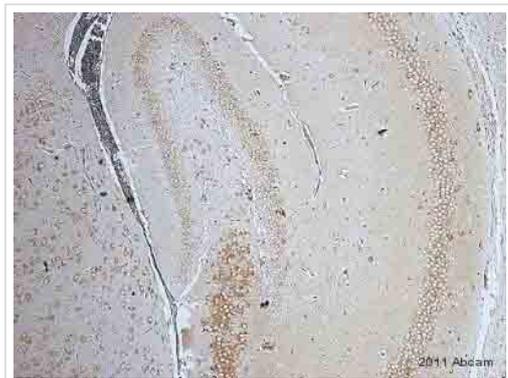


Immunoprecipitation - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

IP with rabbit polyclonal to EZH2 (ab3748).

U2OS cells were transfected with 4ug of Myc-EZH2. WB reprobe using 0.4ug/ml in TBS milk 2%, BSA 0.5%.

NB. ab3748 did not detect endogenous EZH2.

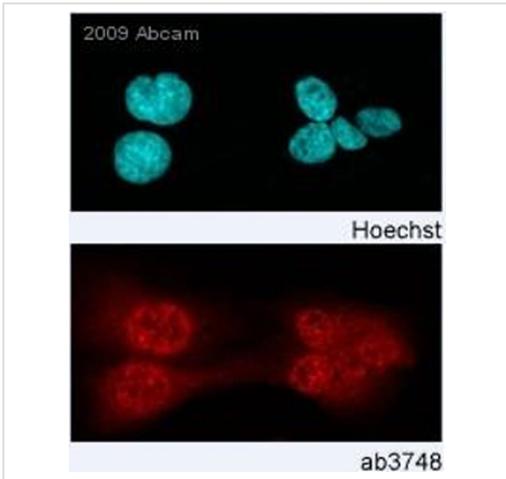


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

Image courtesy of an anonymous Abreview.

ab3748 staining KMT6 / EZH2 in murine hippocampus tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Tissue was fixed in paraformaldehyde and a heat mediated antigen retrieval step was performed using 10mM sodium citrate. Samples were then permeabilized using 0.3% Triton, blocked with 3% BSA for 30 minutes at 22°C and then incubated with ab3748 at a 1/3000 dilution for 16 hours at 4°C. The secondary used was a biotin conjugated horse polyclonal, used at a 1/2000 dilution. ABC method.



Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

This image is a courtesy of Anonymous Abreview

ab3748 staining KMT6 / EZH2 in human U-2 OS cells by Immunocytochemistry/ Immunofluorescence. Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton \times 100 and blocking with 1% BSA was done for 1 hour at RT. Samples were incubated with primary antibody (1/100) for 2 hours. An undiluted Alexa Fluor[®]594-conjugated goat polyclonal to rabbit IgG was used undiluted as secondary antibody.



Western blot - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748) at 1 μ g/ml + EZH2 - Recombinant Protein at 0.1 μ g

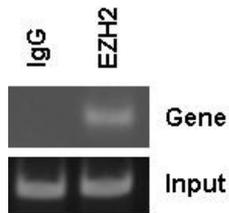
Secondary

Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Observed band size: 40 kDa [why is the actual band size different from the predicted?](#)

ab3748 detects a band at 40 kDa which corresponds to the predicted molecular weight of the Human EZH2 Recombinant Protein.



ChIP analysis of Human glioma cells using ab3748 to bind KMT6 / EZH2. Chromatin was obtained by cross-linking with 1% formaldehyde for 10 minutes and incubated with primary antibody (1/250) for 16 hours at 4°C. Protein binding was detected using semi-quantitative PCR.

ChIP - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

This image is courtesy of an anonymous Abreview

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