


## Product datasheet

# Anti-KMT6 / EZH2 antibody - ChIP Grade ab3748

★★★★☆ 20 Abreviews 54 References 10 Images

### Overview

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<b>Product name</b>	Anti-KMT6 / EZH2 antibody - ChIP Grade
<b>Description</b>	Rabbit polyclonal to KMT6 / EZH2 - ChIP Grade
<b>Host species</b>	Rabbit
<b>Specificity</b>	ab3748 antibody recognises EZH2 in cells transfected with HA-EZH2 in IP.
<b>Tested applications</b>	<b>Suitable for:</b> IHC-FoFr, WB, IP, ChIP, IHC-P, ICC/IF, RIP
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Cow, Human, Xenopus laevis <b>Predicted to work with:</b> Zebrafish 
<b>Immunogen</b>	Synthetic peptide conjugated to KLH derived from within residues 50 - 150 of Human EZH2. Read Abcam's proprietary immunogen policy (Peptide available as <a href="#">ab13744</a> .)
<b>Positive control</b>	WB: Hek293 whole cell lysate; EZH2 recombinant ICC: mouse embryonic stem cells IP: U2OS cells transfected with 4ug of Myc-EZH2
<b>General notes</b>	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

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<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	Preservative: 0.02% Sodium Azide Constituents: 1% BSA, PBS, pH 7.4
<b>Purity</b>	Immunogen affinity purified
<b>Primary antibody notes</b>	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.
<b>Clonality</b>	Polyclonal
<b>Isotype</b>	IgG

### Applications

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

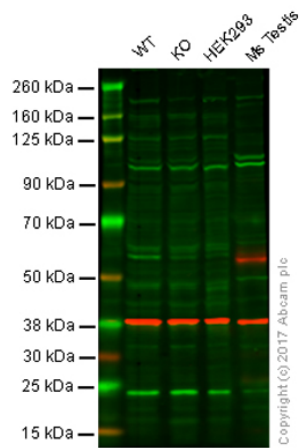
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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 <a href="#">KMT6 / EZH2 peptide (ab13744)</a> .
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

<b>Function</b>	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.
<b>Tissue specificity</b>	Expressed in many tissues. Overexpressed in numerous tumor types including carcinomas of the breast, colon, larynx, lymphoma and testis.
<b>Sequence similarities</b>	Belongs to the histone-lysine methyltransferase family. EZ subfamily. Contains 1 SET domain.
<b>Developmental stage</b>	Expression decreases during senescence of embryonic fibroblasts (HEFs). Expression peaks at the G1/S phase boundary.
<b>Post-translational modifications</b>	Phosphorylated by AKT1. Phosphorylation by AKT1 reduces methyltransferase activity.
<b>Cellular localization</b>	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 1µg/ml and

1/1000 respectively and incubated overnight at 4°C. Blots were

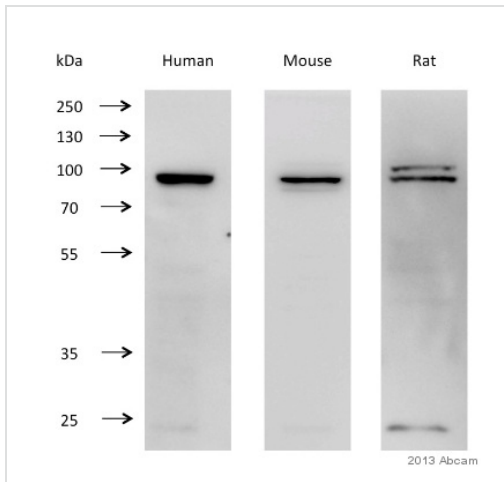
developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW)

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

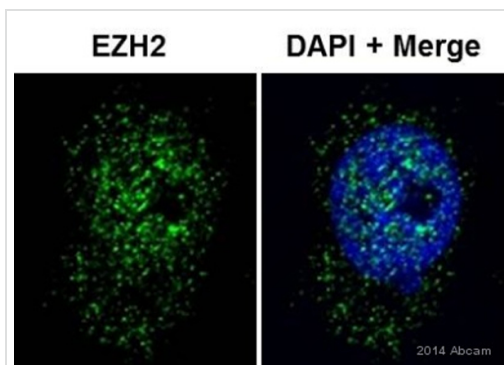
Developed using the ECL technique.

Performed under reducing conditions.

**Observed band size:** 85 kDa

**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

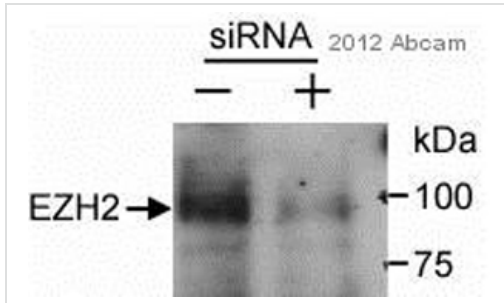
RT. Samples were incubated with primary

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.

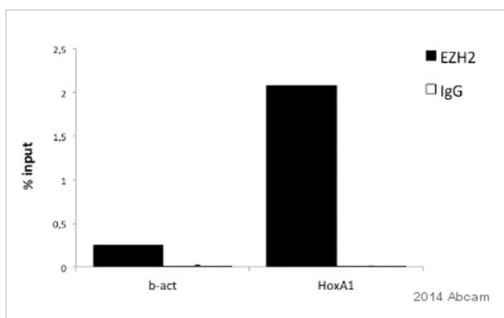
Performed under reducing conditions.

**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.

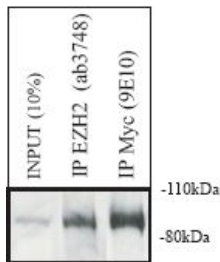


ChIP - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)  
This image is courtesy of an anonymous Abreview

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.

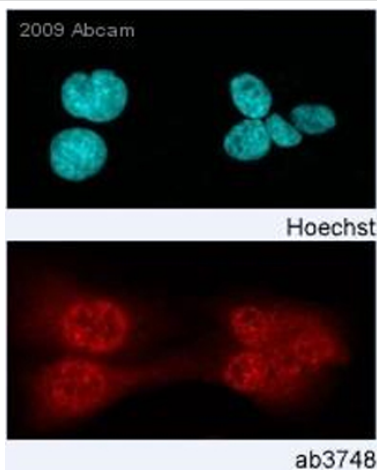


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody - ChIP Grade (ab3748)

Image courtesy of an anonymous Abreview.

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

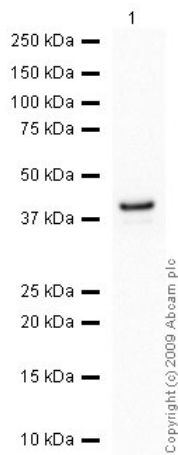
Tissue was fixed in paraformaldehyde and a heat mediated antigen retrieval step was performed using 10mM sodium citrate. Samples were then permeabilized using 0.3% Triton, blocked with 3% BSA for 30 minutes at 22°C and then incubated with ab3748 at a 1/3000 dilution for 16 hours at 4°C. The secondary 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<sup>®</sup>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 µg/ml + EZH2 - Recombinant Protein at 0.1 µg

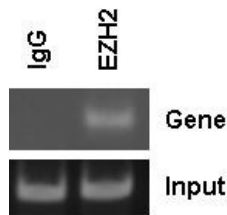
**Secondary**

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

Performed under reducing conditions.

**Observed band size:** 40 kDa

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



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

This image is courtesy of an anonymous Abreview

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.

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