abcam

Product datasheet

Anti-KMT6 / EZH2 antibody [EPR20108] - BSA and Azide free ab240992

KO VALIDATED Recombinant RabMAb

1 References 5 Images

Overview

Product name	Anti-KMT6 / EZH2 antibody [EPR20108] - BSA and Azide free
Description	Rabbit monoclonal [EPR20108] to KMT6 / EZH2 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, ChIP, IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293 and MCF7 whole cell lysates; Wild-type HAP1 cell lysate. IP: HeLa whole cell lysate. ChIP: Chromatin from MDA-MB-231 cells.
General notes	ab240992 is the carrier-free version of **ab191250**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR20108
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab240992 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. Detects a band of approximately 90 kDa (predicted molecular weight: 85 kDa).
ChIP		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.

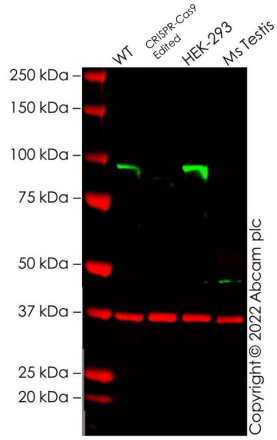
Target

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	Phosphorylated by AKT1. Phosphorylation by AKT1 reduces methyltransferase activity.

modifications

Cellular localization Nucleus.

Images



Western blot - Anti-KMT6 / EZH2 antibody [EPR20108] - BSA and Azide free (ab240992)

All lanes : Anti-KMT6 / EZH2 antibody [EPR20108] - ChIP Grade (**ab191250**) at 1/1000 dilution

Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : ezh2 CRISPR-Cas9 edited MCF7 cell lysate
Lane 3 : HEK-293 cell lysate
Lane 4 : Mouse Testis cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 85 kDa
Observed band size: 90 kDa

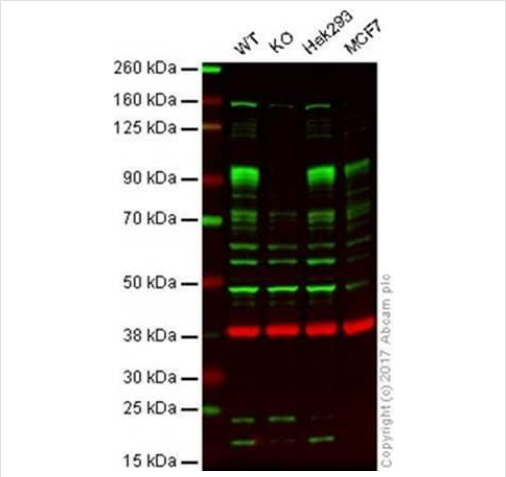
False colour image of Western blot: Anti-KMT6 / EZH2 antibody [EPR20108] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab191250** was shown to bind specifically to KMT6 / EZH2. A band was observed at 90 kDa in wild-type MCF7 cell lysates with no signal observed at this size in ezh2 CRISPR-Cas9 edited cell line **ab281611** (CRISPR-Cas9 edited cell lysate **ab282963**). The band observed in the CRISPR-Cas9 edited lysate lane below 90 kDa is likely to represent a truncated form of KMT6 / EZH2. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and ezh2 CRISPR-Cas9 edited MCF7 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

ChIP - Anti-KMT6 / EZH2 antibody [EPR20108] - BSA and Azide free (ab240992)

Chromatin was prepared from MDA-MB-231 cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of **ab191250** (red), and 20µl of Anti rabbit IgG sepharose beads. 5µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Cell line and primers selection based on literature (PMID: 21880597).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab191250**).



Western blot - Anti-KMT6 / EZH2 antibody [EPR20108] - BSA and Azide free (ab240992)

All lanes : Anti-KMT6 / EZH2 antibody [EPR20108] - ChIP Grade (**ab191250**) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : KMT6 / EZH2 knockout HAP1 cell lysate
Lane 3 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate
Lane 4 : MCF7 (human breast adenocarcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

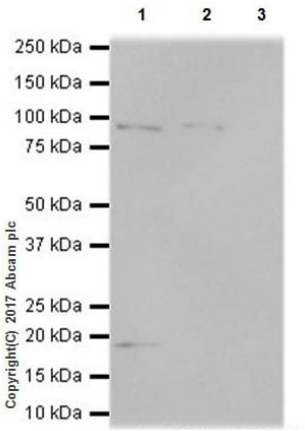
Predicted band size: 85 kDa
Observed band size: 90 kDa

Merged signal (red and green). Green - **ab191250** observed at 93 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab191250 was shown to specifically recognize KMT6/EZH2 in wild-type HAP1 cells along with additional cross-reactive bands. No band was observed when KMT6/EZH2 knockout samples were examined. Wild-type and KMT6/EZH2 knockout samples were subjected to SDS-PAGE. **ab191250** and **ab8245** (loading control to GAPDH) were diluted at 1/1000 and 1/10,000 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 hour at

room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab191250**).



Immunoprecipitation - Anti-KMT6 / EZH2 antibody [EPR20108] - BSA and Azide free (ab240992)

KMT6 / EZH2 was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with **ab191250** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab191250** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: HeLa whole cell lysate 10 µg (Input).

Lane 2: **ab191250** IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab191250** in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab191250**).

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