abcam

ab231165

Anti-KMT6 / EZH2 antibody [EPR9307(2)] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR9307(2)
Purification technique	Affinity purification Protein A
Concentration	1.036 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-

Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-

Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information EZH2

Function	Catalytic subunit of the PRC2/EED-EZH2 complex, a Polycomb group (PcG) complex that methylates 'Lys-9' (H3K9me) and 'Lys-27' (H3K27me) of histone H3, leading to transcriptional repression of the affected target gene (PubMed:14532106, PubMed:15225548, PubMed:15385962, PubMed:16618801, PubMed:16936726, PubMed:17344414, PubMed:22323599, PubMed:24474760, PubMed:26581166, PubMed:30026490, PubMed:30923826). Able to mono-, di- and trimethylate 'Lys-27' of histone H3 to form H3K27me1, H3K27me2 and H3K27me3, respectively (PubMed:15231737, PubMed:17210787, PubMed:18285464, PubMed:22323599, PubMed:30923826). Displays a preference for substrates with less methylation, loses activity when progressively more methyl groups are incorporated into H3K27, H3K27me0 > H3K27me1 > H3K27me2 (PubMed:22323599, PubMed:30923826). Compared to EZH1-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 (PubMed:19026781). The PRC2/EED-EZH2 complex may also serve as a recruiting platform for DNA methyltransferases, thereby linking two epigenetic repression systems (PubMed:16357870, PubMed:17200670). Genes repressed by the PRC2/EED-EZH2 complex include HOXC8, HOXA9, MYT1, CDKN2A and retinoic acid target genes (PubMed:16179254, PubMed:18086877, PubMed:20935635). EZH2 can also methylate non-histone proteins such as the transcription factor GATA4 and the nuclear receptor RORA (PubMed:23063525). Regulates the circadian clock via histone methylation at the promoter of the circadian genes (PubMed:16717091). Essential for the CRY1/2-mediated repression of the transcriptional activation of PER1/2 by the CLOCK-BMAL1 heterodimer; involved in the di and trimethylation of 'Lys-27' of histone H3 on PER1/2 promoters which is necessary for the CRY1/2 proteins to inhibit transcription (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab231165 is the carrier-free version of ab191080.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	KMT6 also known as EZH2 (Enhancer of Zeste Homolog 2) is a protein that functions as a histone methyltransferase. It catalyzes the methylation of histone H3 on lysine 27 (H3K27) a modification associated with gene silencing. EZH2 is a component of the Polycomb Repressive Complex 2 (PRC2) which plays an important role in chromatin remodeling and transcriptional repression. The EZH2 protein has a molecular weight of approximately 87 kDa and its gene is located on chromosome 7. In terms of expression EZH2 is found in various tissues but is notably abundant in growing and dividing cells.
Biological function summary	EZH2 plays a central role in regulating gene expression during development and differentiation. It is a core component of the PRC2 complex which includes other proteins such as EED and SUZ12. Through its catalytic activity EZH2 contributes to the maintenance of the transcriptionally repressed state of genes influencing processes such as cell identity and proliferation. In many cell types EZH2-mediated gene silencing is necessary for normal development and dysregulation can lead to aberrant cellular processes.
Pathways	EZH2 participates in key regulatory networks notably the Wnt signaling pathway and the p53 pathway. Within the Wnt pathway EZH2's activity contributes to controlling gene expression that influences cellular differentiation and proliferation. Meanwhile interaction with the p53 pathway can impact the cellular response to stress and DNA damage highlighting EZH2’s regulatory dimension. Other proteins like β-catenin in the Wnt pathway interact with EZH2 reflecting its integration in these complex signaling networks.

Associated diseases and disorders	EZH2 has been linked to cancer and other proliferative diseases. Overexpression or mutation of EZH2 is frequently observed in cancers such as prostate and breast cancer where it contributes to unchecked cell growth and tumor progression. In these contexts proteins like p53 can interact abnormally with EZH2 leading to failure in tumor suppression mechanisms. Additionally deregulated EZH2 activity is implicated in certain developmental disorders highlighting its broader impact on cellular and organismal homeostasis.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

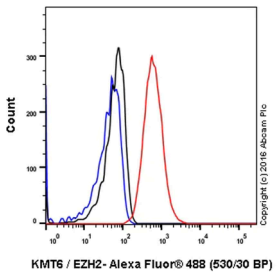
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

5 product images



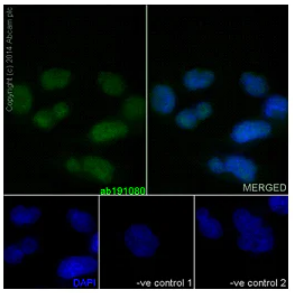
Flow Cytometry (Intracellular) - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - BSA and Azide free (ab231165)

KMT6 / EZH2 Flow Cytometry (Intracellular) staining using rabbit Anti-KMT6 / EZH2 antibody

ab191080 staining KMT6 / EZH2 in the human cell line Jurkat (human acute T cell leukemia) by intracellular flow cytometry. Cells were fixed with 4% paraformaldehyde and the sample was incubated with the primary antibody at a dilution of 1/90. A goat anti rabbit IgG (Alexa Fluor® 488) at a dilution of 1/2000 was used as the secondary antibody.
Isoytype control: Rabbit monoclonal IgG (Black)

Unlabelled control: Cell without incubation with primary antibody and secondary antibody (Blue)

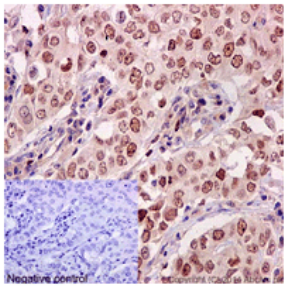
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191080).



Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - BSA and Azide free (ab231165)

KMT6 / EZH2 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-KMT6 / EZH2 antibody

Immunofluorescent analysis of HeLa cells (4% Paraformaldehyde-fixed; 0.1% tritonX-100-permeabilized) labeling KMT6 / EZH2 with ab191080 at 1/250 dilution followed by Goat anti rabbit IgG (AlexaFluor® 488) secondary at 1/200 dilution and counter-stained with DAPI (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab191080).

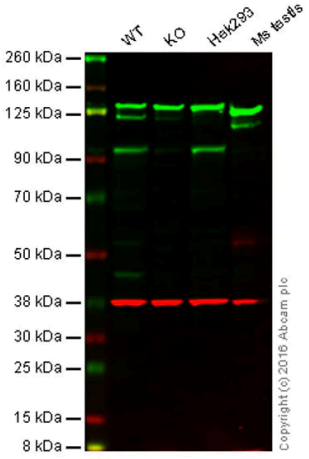


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - BSA and Azide free (ab231165)

KMT6 / EZH2 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-KMT6 / EZH2 antibody

This IHC data was generated using the same anti-KMT6/EZH2 antibody clone, EPR9307(2), in a different buffer formulation (cat# ab191080).
Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling KMT6 / EZH2 with ab191080 at 1/250 dilution followed by pre-diluted HRP Polymer for Rabbit IgG secondary antibody and counter-stained with Hematoxylin (inset: negative control).

Heat mediated antigen retrieval was performed with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Western blot - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - BSA and Azide free (ab231165)

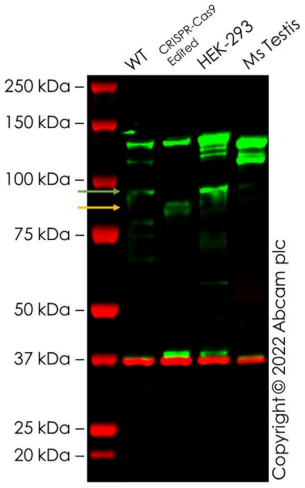
KMT6 / EZH2 Western blot staining using rabbit Anti-KMT6 / EZH2 antibody

This WB data was generated using the same anti-KMT6/EZH2 antibody clone, EPR9307(2), in a different buffer formulation (cat# ab191080).
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: KMT6 / EZH2 knockout HAP1 cell lysate (20 µg)
Lane 3: HEK293 cell lysate (20 µg)
Lane 4: Ms testis tissue lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab191080 observed at 93 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab191080 was shown to recognize KMT6 / EZH2 when KMT6 / EZH2 knockout samples were used, along with additional cross-reactive bands. Wild-type and KMT6 / EZH2 knockout samples were subjected to SDS-PAGE. ab191080 and ab8245 (loading control to GAPDH) were diluted at 1/500 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 h at room temperature before imaging.

All lanes:
Western blot - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080)

Predicted band size: 85 kDa, 92 kDa



Western blot - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - BSA and Azide free (ab231165)

False colour image of Western blot: Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal staining at 1/500 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab191080 was shown to bind specifically to KMT6 / EZH2. A band was observed at 90 kDa (green arrow) 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 (yellow arrow) 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.
All lanes:
Western blot - Anti-KMT6 / EZH2 antibody [EPR9307(2)] - N-terminal (ab191080) at 1/1000 dilution

Lane 1:
Wild-type MCF7 cell lysate at 20 µg

Lane 2:
ezh2 CRISPR-Cas9 edited MCF7 cell lysate at 20 µg

Lane 3:
HEK-293 cell lysate at 20 µg

Lane 4:
Mouse Testis cell lysate at 20 µg

Performed under reducing conditions.

Observed band size: 90 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.