abcam

ab245738

Anti-KMT6 / EZH2 antibody [SP129] - BSA and Azide free

Recombinant | RabMAb | Advanced Validation

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	SP129
Purification technique	Affinity purification Protein A/G
Concentration	1.003 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purified from TCS by protein A/G.

Reactivity data

ChIC/CUT&RUN-seq

Tested

Species	Human

Dilution info	-
Notes	-

Expected

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

Predicted

Species	Rat, Monkey
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

Predicted

Species	Rat, Monkey
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	Primary antibody incubation for 30 minutes at 4 degrees.

Expected

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

Predicted

Species	Rat, Monkey
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	-
Notes	Primary antibody incubation for 1 hour at room temperature.

Species	Human
Dilution info	-
Notes	Primary antibody incubation for 1 hour at room temperature.

Predicted

Species	Rat, Monkey
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	Perform heat mediated antigen retrieval with EDTA buffer before commencing with IHC staining protocol.Primary antibody incubation for 30 minutes at room temperature.

Expected

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

Predicted

Species	Rat, Monkey
Dilution info	-
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

ab245738 is the carrier-free version of ab227648.

Patented technology

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?

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 batch production

For more information, read more on recombinant antibodies.

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.

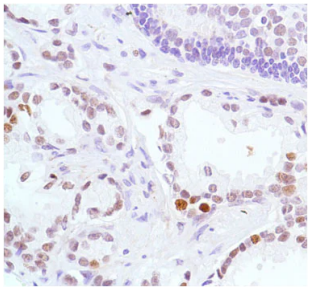
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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5 product images

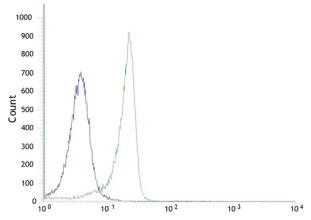


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 antibody [SP129] - BSA and Azide free (ab245738)

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

Formalin-fixed, paraffin-embedded human prostate cancer tissue stained for KMT6 / EZH2 using ab227648 at 1/100 dilution in immunohistochemical analysis.

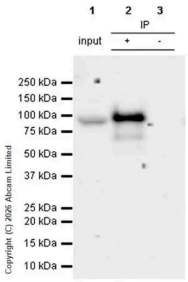
This data was developed using the same antibody clone in a different buffer formulation containing PBS#44; BSA#44; and sodium azide (ab227648)



Flow Cytometry (Intracellular) - Anti-KMT6 / EZH2 antibody [SP129] - BSA and Azide free (ab245738)

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

Flow cytometric analysis of HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling KMT6 / EZH2 with ab227648 at 1/100 dilution (green) compared with a negative control of rabbit IgG (blue).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (ab227648).



Immunoprecipitation - Anti-KMT6 / EZH2 antibody [SP129] - BSA and Azide free (ab245738)

This data was developed using ab227648, the same antibody clone in a different buffer formulation.

KMT6 / EZH2 was immunoprecipitated from 0.35mg lysate HEK-293 (human embryonic kidney epithelial cell) whole cell lysate with ab227648 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab227648 at 1/1000 dilution. Capture antibody, 1:30 dilution (2µg in 0.35mg lysates) VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

To minimize protein degradation, cells were lysed immediately after harvest and then applied for Immunoprecipitation as soon as possible.

Rabbit monoclonal IgG (ab172730) Isotype Control was used instead of ab185971 in HEK-293 whole cell lysate (Lane 3).

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

To minimize protein degradation, cells were lysed immediately after harvest and then applied for Immunoprecipitation as soon as possible.

All lanes:
Immunoprecipitation - Anti-KMT6 / EZH2 antibody [SP129] (ab227648) at 1/30 dilution

Lane 1:
HEK-293 (human embryonic kidney epithelial cell) whole cell lysate 10 µg

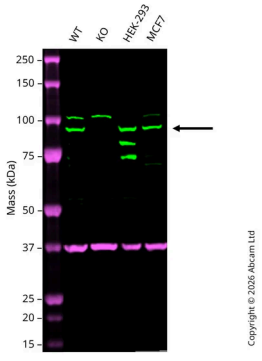
Lane 2:
HEK-293 (human embryonic kidney epithelial cell) whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Observed band size: 85 kDa

Exposure time: 32s



Western blot - Anti-KMT6 / EZH2 antibody [SP129] - BSA and Azide free (ab245738)

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab227648).

Western blot: Anti-KMT6 / EZH2 antibody [SP129] ab227648 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH ab8245 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 85 kDa in Wild-type A375 cell lysates with no signal observed at this size in EZH2 knockout A375 cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5% 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 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:

Western blot - Anti-KMT6 / EZH2 antibody [SP129] (ab227648) at 1/1000 dilution

Lane 1:
Wild-type A375 cell lysates at 20 µg

Lane 2:
EZH2 knockout A375 cell lysates at 20 µg

Lane 3:
HEK-293 cell lysates at 20 µg

Lane 4:
MCF7 cell lysates at 20 µg

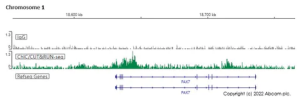
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 85 kDa

Observed band size: 85 kDa



ChIC/CUT&RUN sequencing - Anti-KMT6 / EZH2 antibody [SP129] - BSA and Azide free (ab245738)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2 x 10^5 K-562 (Human chronic myelogenous leukemia lymphoblast) cells and 5 µg of ab227648 [SP129]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control ab172730 is also shown. Additional screenshots of mapped reads can be found in the Protocol booklet in the Product Protocol section. The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA and sodium azide (ab227648).

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