abcam

ab300491

Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR24904-99
Purification technique	Affinity purification Protein A
Concentration	0.469 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/200
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Dot

Tested

Species	Human
Dilution info	1/1000
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

IP

Tested

Species	Human

Dilution info	1/30
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information EZH2 Y646N

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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks

Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

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

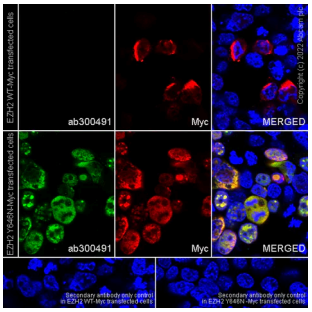
We do not recommend this combination. It is not covered by our product promise.

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.

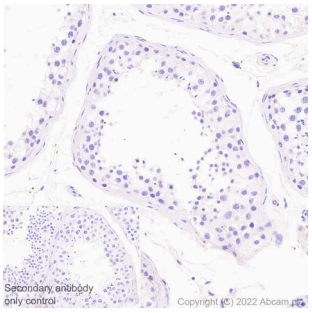
Full details and terms and conditions can be found here:
Terms & Conditions.

7 product images



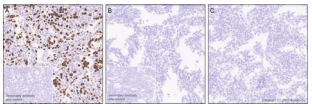
Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HEK293T (human embryonic kidney epithelial cell) cells labelling KMT6 / EZH2 (mutated Y646N) with ab300491 at 1/50 (9.38ug/mL) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/mL dilution (Green). Confocal image showing nuclear staining in HEK-293T cells transfected with a human EZH2 Y646N expression vector containing a myc tag. is observed. Myc-Tag Mouse mAb (Alexa Fluor® 647) was used to counterstain tubulin at 1/100 0.38ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/mL dilution.



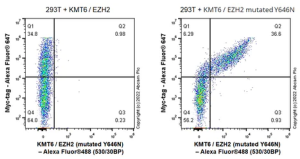
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling KMT6 / EZH2 (mutated Y646N) with ab300491 at 1/200 (2.345 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Negative control: No staining on human testis. The section was incubated with ab300491 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used.Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



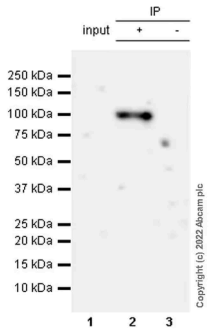
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491)

Immunohistochemical analysis of paraffin-embedded Panel A HEK-293T ce tissue labeling KMT6 / EZH2 (mutated Y646N) with ab300491 at 1/200 (2.345 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Positive staining on HEK-293T cells transfected with a human EZH2 Y646N expression vector (Panel A), no staining on HEK-293T cells transfected with a human EZH2 WT expression vector (Panel B), no staining on HEK-293T transfected with an empty vector (Panel C). The section was incubated with ab300491 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used.Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491)

Flow Cytometry (Intracellular) analysis of HEK-293T (human embryonic kidney) cells transfected with a human wild type KMT6 / EZH2 expression vector containing a myc-his tag (Left) / HEK-293T transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag (Right). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% Methanol. ab300491 used at a 1/500 dilution. A Goat Anti-Rabbit IgG (Alexa Fluor® 488, (ab150081)) was used as the secondary antibody at a 1/2000 dilution.



Immunoprecipitation - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491)

KMT6 / EZH2 (mutated Y646N) was immunoprecipitated from 0.35 mg HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag, whole cell lysate 10 ug with ab300491 at 1/30 dilution (2ug in 0.35mg lysates).
Western blot was performed on the immunoprecipitate using ab300491 at 1/1000 dilution.

VeriBlot for IP secondary antibody (HRP) (ab131366) was used at 1/5000 dilution.

Lane 1: HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag, whole cell lysate 10 ug

Lane 2: ab300491 IP in HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag, whole cell lysate

Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab300491 in HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag, whole cell lysate

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

This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

All lanes:
Immunoprecipitation - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491) at 1/1000 dilution

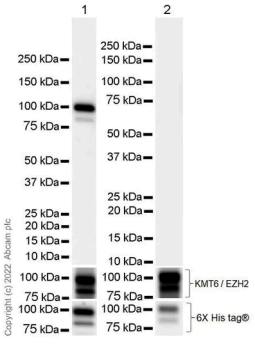
All lanes:
HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag, whole cell lysate at 10 µg

Secondary

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

Developed using the ECL technique.

Exposure time: 3min



Western blot - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491)

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
This blot was developed using a high sensitivity ECL substrate. The high-sensitivity ECL substrate used allows for the detection of proteins in the mid-femtogram range.

All lanes:
Western blot - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491) at 1/1000 dilution

Lane 1:
HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containi a myc-his tag, whole cell lysate, 10

Lane 2:
HEK-293T cells transfected with a human wild type KMT6 / EZH2 expression vector containi a myc-his tag, whole cell lysate, 10

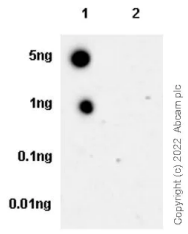
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

Observed band size: 98 kDa

Exposure time: 6s



Dot Blot - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (ab300491)

Dot blot analysis of KMT6 / EZH2 (mutated Y646N) using ab300491 at 1:1000 (0.469 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1:100,000 dilution. Exposure time: 3 minutes Blocking and diluting buffer and concentration: 5% NFDM/TBST

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