

Product datasheet

Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free) ab300492

Recombinant RabMAb

8 Images

Overview

Product name	Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free)
Description	Rabbit monoclonal [EPR24904-99] to KMT6 / EZH2 (mutated Y646N) - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), IP, WB, Dot blot, IHC-P, ICC/IF
Species reactivity	Reacts with: Human Does not react with: Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag, whole cell lysate. Dot Blot: Human KMT6 / EZH2 mutated Y646N peptide. IHC-P: Transfected HEK-293T cells. ICC/IF: Transfected HEK-293T cells. Flow Cyt (Intra): Transfected HEK-293T cells. IP: Transfected HEK-293T cells.
General notes	ab300492 is a carrier free version of ab300491 .

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.
Storage buffer	pH: 7.20 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR24904-99
Isotype	IgG

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab300492 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
Flow Cyt (Intra)		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 85 kDa.
Dot blot		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		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.

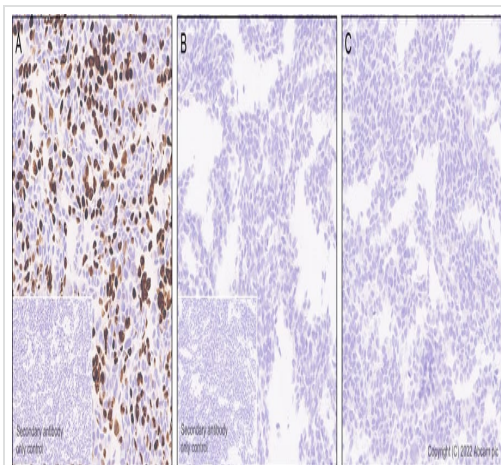
Post-translational modifications

Phosphorylated by AKT1. Phosphorylation by AKT1 reduces methyltransferase activity.

Cellular localization

Nucleus.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free) (AB300492)

This data was developed using **ab300491**, the same antibody clone in a different buffer formulation.

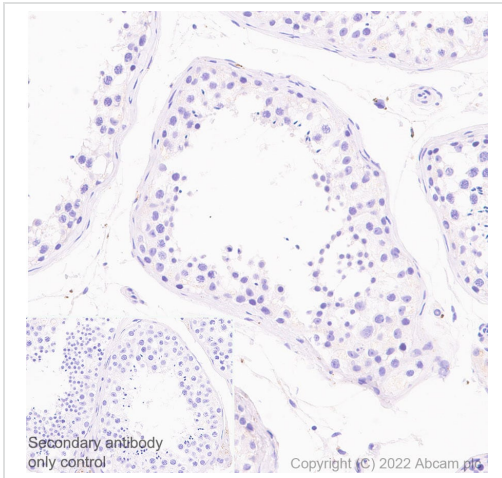
Immunohistochemical analysis of paraffin-embedded sections labeling KMT6 / EZH2 (mutated Y646N) with **ab300491** at 1/200 dilution (2.345 µg/ml) followed by a ready to use Leica DS9800 (BOND™ Polymer Refine Detection) kit.

Panel A: HEK-293T cells transfected with a human EZH2 Y646N expression vector containing a myc-His tag.

Panel B: HEK-293T cells transfected with a human EZH2 WT expression vector containing a myc-His tag.

Panel C: HEK-293T transfected with an empty vector.

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). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



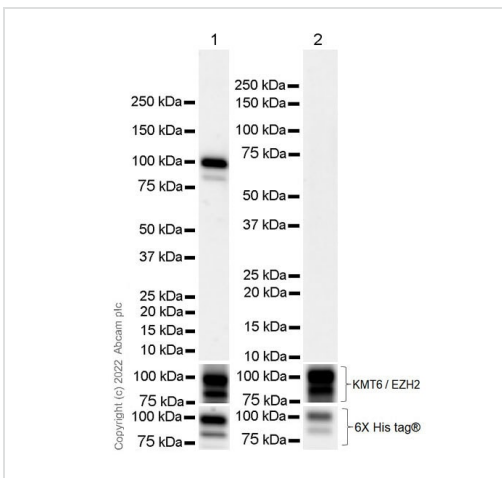
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free) (AB300492)

This data was developed using **ab300491**, the same antibody clone in a different buffer formulation.

Immunohistochemical analysis of paraffin-embedded human testis tissue labeling KMT6 / EZH2 (mutated Y646N) with **ab300491** at 1/200 dilution (2.345 µg/ml) followed by a ready to use Leica DS9800 (BOND™ Polymer Refine Detection) kit.

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). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Western blot - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free) (AB300492)

All lanes : 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 containing a myc-his tag, whole cell lysate

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

Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 85 kDa

Observed band size: 98 kDa

Exposure time: 6 seconds

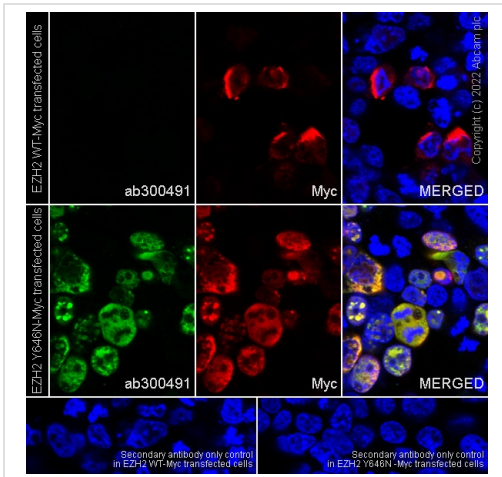
This data was developed using [ab300491](#), the same antibody clone in a different buffer formulation.

Blocking / Diluting buffer: 5% NFDN/TBST.

This blot was developed using a high sensitivity ECL substrate.

Loading controls

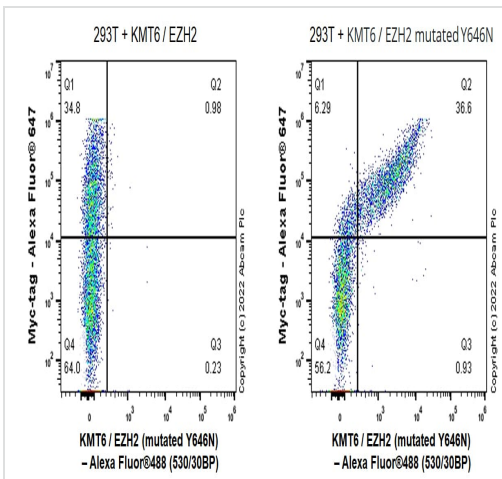
All Lanes: [ab191250](#), Recombinant Anti-KMT6 / EZH2 antibody and [ab213204](#), Recombinant Anti-6X His tag® antibody



Immunocytochemistry/ Immunofluorescence - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free) (ab300492)

This data was developed using [ab300491](#), the same antibody clone in a different buffer formulation.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK293T (human embryonic kidney epithelial cells) labelling KMT6 / EZH2 (mutated Y646N) with [ab300491](#) at 1/50 dilution followed by [ab150081](#) Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/2000 dilution (green). Confocal image showing nuclear staining in HEK-293T cells transfected with a human EZH2 Y646N expression vector containing a myc tag. An anti Myc-Tag Mouse monoclonal antibody (Alexa Fluor® 647) at 1/100 dilution was used as a counterstain (red). The nuclear counterstain is DAPI (blue).

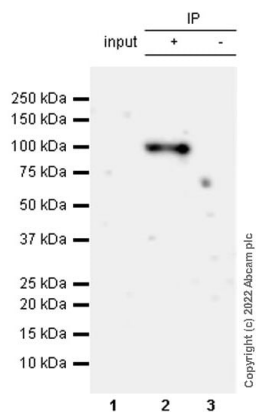


Flow Cytometry (Intracellular) - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free) (AB300492)

This data was developed using [ab300491](#), the same antibody clone in a different buffer formulation.

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] (BSA and Azide free) (AB300492)

This data was developed using **ab300491**, the same antibody clone in a different buffer formulation.

KMT6 / EZH2 (mutated Y646N) was immunoprecipitated from 10 µg HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag, whole cell lysate with **ab300491** at 1/30 dilution. Western blot was performed from the immunoprecipitate using **ab300491** at 1/1000 dilution. VeriBlot for IP secondary antibody (HRP) (**ab131366**), was used as secondary antibody 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 µg (Input).

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

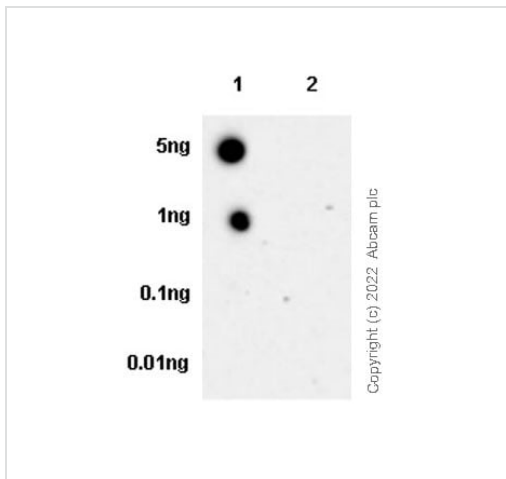
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab300491** in HEK-293T cells transfected with a human KMT6 / EZH2 mutated Y646N expression vector containing a myc-his tag, whole cell lysate.

Blocking and dilution buffer: 5% NFDm/TBST.

Exposure time: 3 minutes.

Observed MW(KDa): 98

This blot was developed using a high sensitivity ECL substrate.



Dot Blot - Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free) (AB300492)

This data was developed using **ab300491**, the same antibody clone in a different buffer formulation.

Dot Blot - Anti KMT6 / EZH2 (mutated Y646N)

Concentration of **ab300491**: 1/1000 dilution (0.469 µg/ml)

Secondary ab: Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**), 1/100000 dilution





Blocking/diluting buffer: 5% NFD/MTBST

Lane 1: Human KMT6 / EZH2 mutated Y646N peptide

Lane 2: Human wild type KMT6 / EZH2 peptide

Exposure time: 3 minutes

Why choose a recombinant antibody?

 <p>Research with confidence Consistent and reproducible results</p>	 <p>Long-term and scalable supply Recombinant technology</p>
 <p>Success from the first experiment Confirmed specificity</p>	 <p>Ethical standards compliant Animal-free production</p>

Anti-KMT6 / EZH2 (mutated Y646N) antibody [EPR24904-99] (BSA and Azide free) (AB300492)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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