abcam

Product datasheet

Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade - BSA and Azide free ab240310

Recombinant RabMAb

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Overview

Product name	Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade - BSA and Azide free
Description	Rabbit monoclonal [EPR18340] to Histone H3 (mutated K27M) - ChIP Grade – BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ChIP, ICC/IF, IP, Indirect ELISA
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: His-tagged recombinant histone H3 K27M protein, His-tagged recombinant wild type histone H3 protein; HEK-293 transfected with Histone H3 (K27M) expression vector containing a myc-His-tag®, whole cell lysate. IP: HEK-293T transfected with myc-tagged H3 (K27M) expression vector whole cell lysate. ICC/IF: HEK-293T transfected with myc-tagged H3 (K27 mutated to M) expression vector. ChIP: Chromatin prepared from HEK-293T transfected with myc-tagged H3 (K27 mutated to M) expression vector.
General notes	ab240310 is the carrier-free version of **ab190631**. 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	EPR18340
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab240310 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

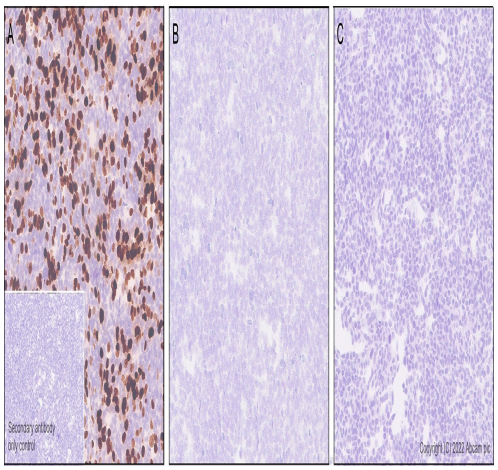
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
IHC-P		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
Indirect ELISA		Use at an assay dependent concentration.

Target

Function	Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA accessibility is regulated via a complex set of post-translational modifications of histones, also called histone code, and nucleosome remodeling.
Sequence similarities	Belongs to the histone H3 family.

Developmental stage	Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.
Post-translational modifications	Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me). Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PADI4 impairs methylation and represses transcription. Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation. Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression. Asymmetric dimethylation at Arg-3 (H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3). H3R2me2a is present at the 3' of genes regardless of their transcription state and is enriched on inactive promoters, while it is absent on active promoters. Methylation at Lys-5 (H3K4me), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are linked to gene repression. Methylation at Lys-10 (H3K9me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) and prevents subsequent phosphorylation at Ser-11 (H3S10ph) and acetylation of H3 and H4. Methylation at Lys-5 (H3K4me) and Lys-80 (H3K79me) require preliminary monoubiquitination of H2B at 'Lys-120'. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin. Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes following external stimulation, like mitogens, stress, growth factors or UV irradiation and result in the activation of genes, such as c-fos and c-jun. Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at Lys-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation at Ser-11 (H3S10ph) is also an essential regulatory mechanism for neoplastic cell transformation. Phosphorylated at Ser-29 (H3S28ph) by MLTK isoform 1, RPS6KA5 or AURKB during mitosis or upon ultraviolet B irradiation. Phosphorylation at Thr-7 (H3T6ph) by PRKCBB is a specific tag for epigenetic transcriptional activation that prevents demethylation of Lys-5 (H3K4me) by LSD1/KDM1A. At centromeres, specifically phosphorylated at Thr-12 (H3T11ph) from prophase to early anaphase, by DAPK3 and PKN1. Phosphorylation at Thr-12 (H3T11ph) by PKN1 is a specific tag for epigenetic transcriptional activation that promotes demethylation of Lys-10 (H3K9me) by KDM4C/JMJD2C. Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin. Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination (By similarity). Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins.
Cellular localization	Nucleus. Chromosome.

Images



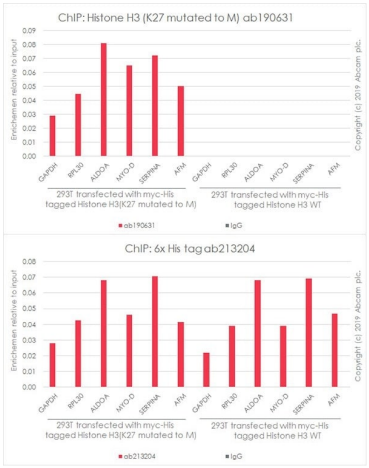
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade - BSA and Azide free (ab240310)

This data was developed using **ab190631**, the same antibody clone in a different buffer formulation.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of tissue sections from(A) HEK-293T transfected with Histone H3 (mutated K27M) expression vector containing a his tag. (B) HEK-293T transfected with wildtype Histone H3 expression vector containing a his tag. (C) HEK-293T transfected with empty vector containing a his tag, labeling Histone H3 (mutated K27M) with **ab190631** at 1/1000 dilution and ready to use secondary LeicaDS9800 (Bond™ Polymer Refine Detection) counterstained with Hematoxylin.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins

Positive staining on HEK-293T transfected with Histone H3 (mutated K27M) expression vector cell pellets (image A), no staining on HEK-293T transfected with wildtype Histone H3 expression vector cell pellets (Image B) or empty vector cell pellets (Image C).
The section was incubated with **ab190631** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument



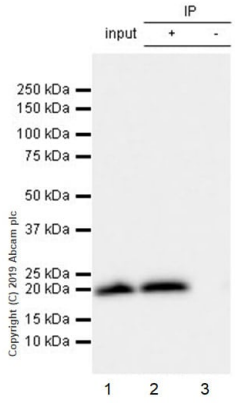
ChIP - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade - BSA and Azide free (ab240310)

This data was developed using **ab190631**, the same antibody clone in a different buffer formulation.

Chromatin was prepared from 293T transfected with myc-His tagged Histone H3(K27 M) and Histone H3 WT cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30mins and then formaldehyde for 10min.
The ChIP was performed with 25 µg of chromatin, 2 µg of **ab190631** (red), 2 µg of **ab213204** (red) (bottom panel, served as internal control) or 2 µg of rabbit normal IgG **ab172730** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).

Primers and probes are located in the first kb of the transcribed region.

***https://www.abcam.com/resources?keywords=X%20ChIP%20protocol**



Immunoprecipitation - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade - BSA and Azide free (ab240310)

Histone H3 (K27 mutated to M) was immunoprecipitated from 0.35 mg 293T (human embryonic kidney epithelial cell) cells transfected with myc-tagged H3(K27M) expression vector whole cell lysate 10ug with **ab190631** at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using **ab190631** at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: 293T (human embryonic kidney epithelial cell) cells transfected with myc-tagged H3(K27M) expression vector whole cell lysate 10ug

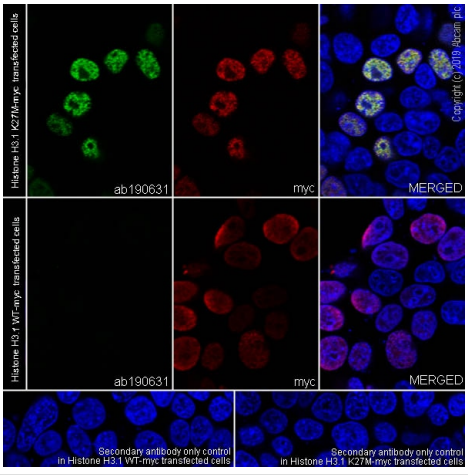
Lane 2: **ab190631** IP in 293T cells transfected with myc-tagged H3(K27M) expression vector whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab190631** in 293T cells transfected with myc-tagged H3(K27M) expression vector whole cell lysate

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

Exposure time: 5 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, azide, BSA and glycerol (**ab190631**).

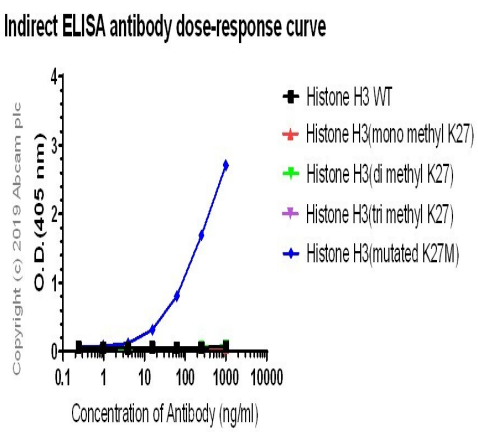


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade - BSA and Azide free (ab240310)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized 293T (human embryonic kidney epithelial cell) cells labelling Histone H3 (K27 mutated to M) with **ab190631** at 1/5000 (0.2 ug/ml) dilution, followed by **ab190631** anti- H3(K27 mutated to M) **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing nuclear staining in 293T cells transfected with myc-tagged H3 (K27 mutated to M) expression vector. is observed. **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is Ab190631 anti- H3(K27 mutated to M) **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 ug/ml) dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab190631**).



Indirect ELISA - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade - BSA and Azide free (ab240310)

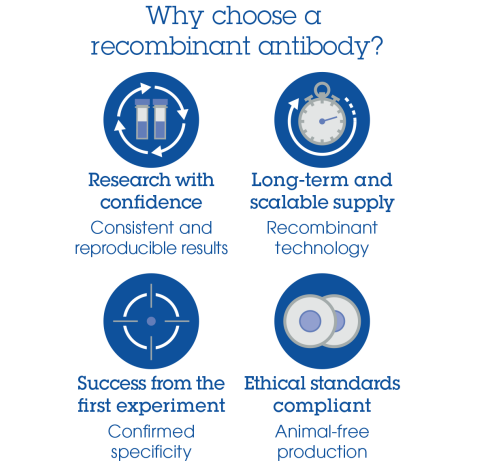
This data was developed using **ab190631**, the same antibody clone in a different buffer formulation.

ELISA using **ab190631** at varying antibody concentrations and antigen concentration at 1000 ng/mL. An Alkaline Phosphatase-conjugated Goat Anti-Rabbit IgG (H+L) (1/2500) was used as the secondary antibody.

The blue line indicates binding to the Histone H3 (mutated K27M) peptide. Binding to the following peptides was not seen:

Histone H3 WT,
Histone H3 (mono methyl K27),
Histone H3 (di methyl K27),
Histone H3 (tri methyl K27).

This indicates the specificity of **ab190631** for mutated K27M of Histone H3.



Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade - BSA and Azide free (ab240310)

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