abcam

Product datasheet

Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade ab190631

リコンビナント RabMAb

4 Abreviews 9 References 画像数 10

製品の概要

製品名	Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR18340] to Histone H3 (mutated K27M) - ChIP Grade
由来種	Rabbit
アプリケーション	**適用あり:** WB, Indirect ELISA, IHC-P, ICC/IF, IP, ChIP
種交差性	**交差種:** Mouse, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR18340
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab190631の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★★★ (2)	1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
Indirect ELISA		Use a concentration of 1 µg/ml.
IHC-P	★★★★★ (1)	1/500.
ICC/IF	★★★★★ (1)	1/5000.
IP		1/30.
ChIP		Use 2 µg for 25 µg of chromatin.

ターゲット情報

機能

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.

配列類似性

Belongs to the histone H3 family.

発生段階

Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

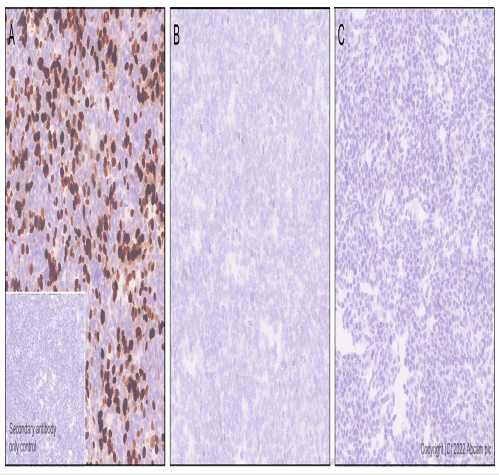
翻訳後修飾

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.

細胞内局在 Nucleus. Chromosome.

画像



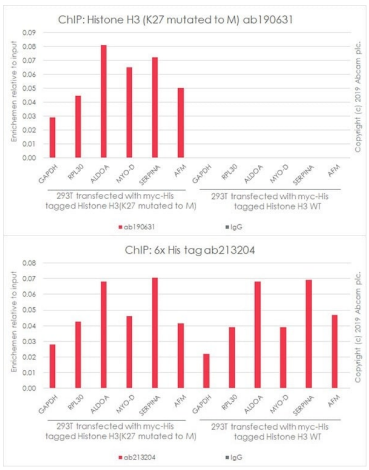
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

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

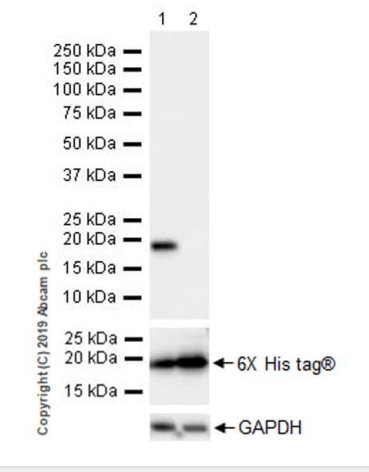


ChIP - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

Chromatin was prepared from HEK-293T transfected with myc-His tagged Histone H3 (K27M) 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**



Western blot - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

All lanes : Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631) at 1/1000 dilution

Lane 1 : HEK-293 transfected with Histone H3.1 (K27M) expression vector containing a myc-His-tag, whole cell lysate
Lane 2 : HEK-293 transfected with Histone H3(WT) expression vector containing a myc-His-tag, whole cell lysate

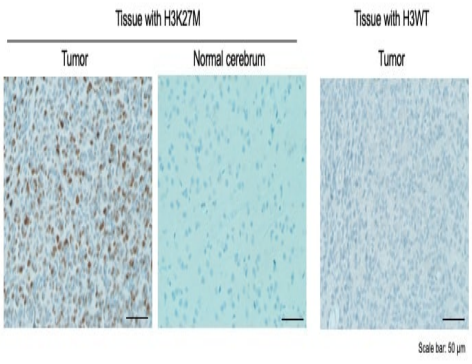
Lysates/proteins at 10 µg per lane.

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

Predicted band size: 15 kDa
Observed band size: 18 kDa

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

Exposure time: 6 seconds.

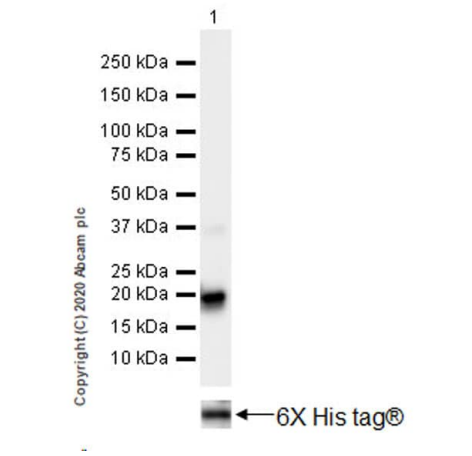


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

This image is courtesy of Dr Yusuke Tomita, Northwestern University.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of tissue sections from mouse brain tumor with and without H3K27M mutation labeling Histone H3 (mutated K27M) with ab190631 at 1/500 dilution (1 hour at 36°C in 2% BSA in PBS).

Tissue was fixed with 20% Neutral Buffer Formalin for 48 hours, then changed to 70% ethanol for a week. Paraffin-embedded tissue was cut in 5 micrometer thickness with microtome. Antigen retrieval was done with Cell-Conditioning 1 (Roche) at 95 degree. A HRP conjugated monoclonal antibody (DISCOVERY UltraMap anti-Rb HRP (RUO)) was used as the secondary antibody.



Western blot - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631) at 1/1000 dilution + HEK-293 transfected with Histone H3.3 (K27M) expression vector containing a myc-His-tag®, whole cell lysate at 10 µg

Secondary

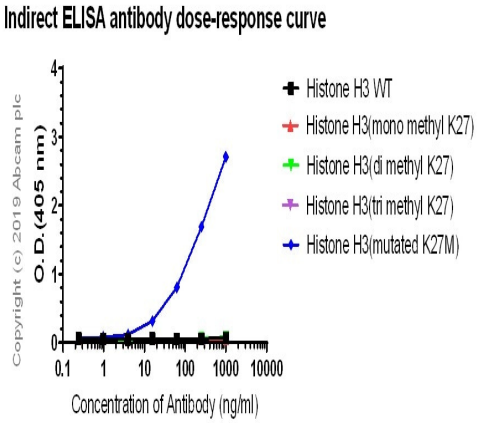
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 15 kDa

Observed band size: 18 kDa

Exposure time: 3 seconds

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



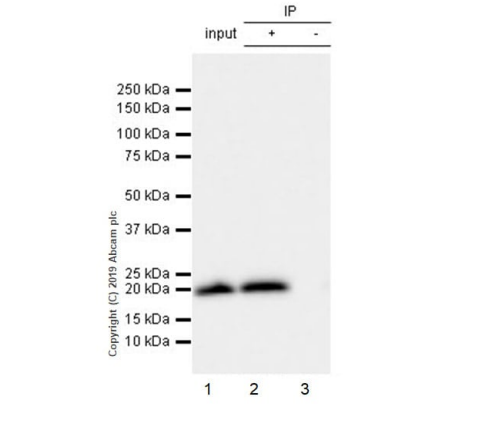
Indirect ELISA - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

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.



Immunoprecipitation - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

Histone H3 (K27 mutated to M) was immunoprecipitated from 0.35 mg HEK-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.

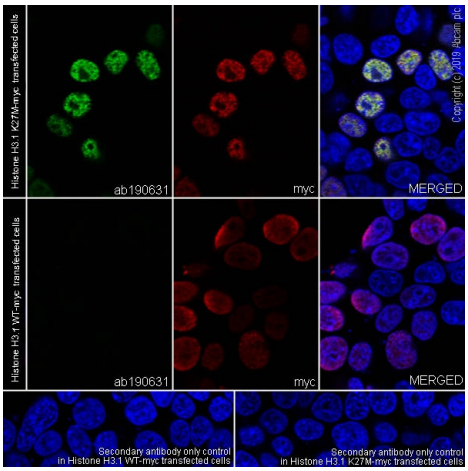
Lane 1: HEK-293T (human embryonic kidney epithelial cell) cells transfected with myc-tagged H3(K27M) expression vector whole cell lysate 10ug

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

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

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

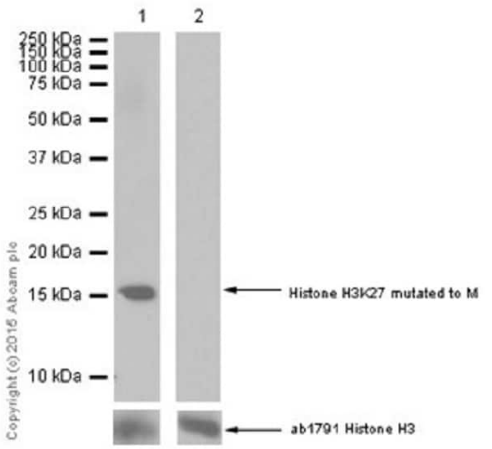
Exposure time: 5 seconds



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (human embryonic kidney epithelial cell) cells labeling Histone H3 (K27 mutated to M) with ab190631 at 1/5000 (0.2 ug/ml) dilution, followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 (2 ug/ml) dilution (Green). Confocal image showing nuclear staining in HEK-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.



Western blot - Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631)

All lanes : Anti-Histone H3 (mutated K27M) antibody [EPR18340] - ChIP Grade (ab190631) at 1/1000 dilution

Lane 1 : His-tagged recombinant histone H3 K27M protein
Lane 2 : His-tagged recombinant wild type histone H3 protein

Lysates/proteins at 0.01 µg per lane.

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

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 15 seconds

Blocking/Dilution buffer: 5% BSA/TBST.

Anti-Histone H3 (mutated K27M) antibody

[EPR18340] - ChIP Grade (ab190631)

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