

Product datasheet

Anti-Histone H3 (mono methyl K9) antibody [EPR16989] - ChIP Grade ab176880

Recombinant RabMAb

★★★★☆ [3 Abreviews](#) [8 References](#) [8 Images](#)

Overview

Product name	Anti-Histone H3 (mono methyl K9) antibody [EPR16989] - ChIP Grade
Description	Rabbit monoclonal [EPR16989] to Histone H3 (mono methyl K9) - ChIP Grade
Host species	Rabbit
Tested applications	Suitable for: PepArr, ChIP, WB, IHC-P, ICC/IF
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa and NIH/3T3 whole cell lysates. IHC-P: Human colon, mouse colon and rat pancreas tissues. ICC/IF: HeLa cells. ChIP: Chromatin was prepared from HeLa cells.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none">- High batch-to-batch consistency and reproducibility- Improved sensitivity and specificity- Long-term security of supply- Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16989
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab176880 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
PepArr		Use at an assay dependent concentration.
ChIP		Use 2 µg for 25 µg of chromatin.
WB	★★★★★ (3)	1/20000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/2000.

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 PAD4 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.

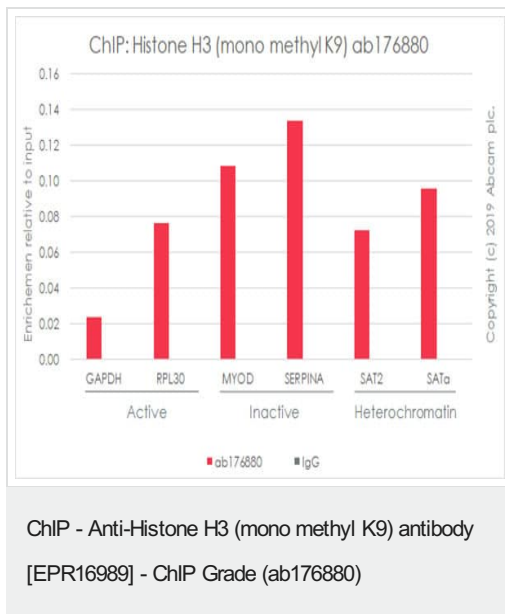
Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are linked to gene repression. Methylation at Lys-10 (H3K9me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) 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.

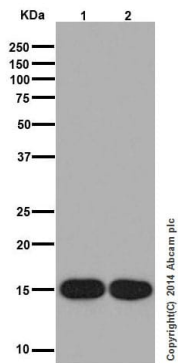
Cellular localization

Nucleus. Chromosome.

Images



Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab176880 (red), and 20µl of Protein A/G sepharose beads. Rabbit normal IgG was added to the beads control (grey). 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.



Western blot - Anti-Histone H3 (mono methyl K9) antibody [EPR16989] - ChIP Grade (ab176880)

All lanes : Anti-Histone H3 (mono methyl K9) antibody [EPR16989]
- ChIP Grade (ab176880) at 1/20000 dilution

Lane 1 : HeLa whole cell lysates

Lane 2 : NIH/3T3 whole cell lysates

Lysates/proteins at 10 µg per lane.

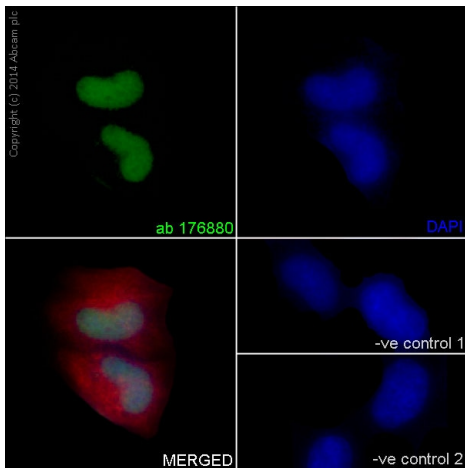
Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 15 kDa

Observed band size: 15 kDa

Blocking/Dilution buffer: 5% NFDm/TBST.

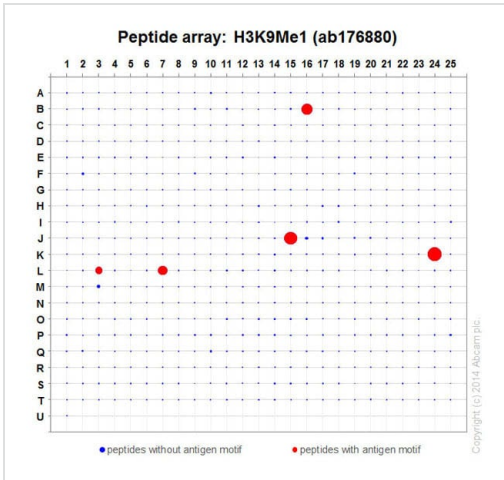


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K9) antibody [EPR16989] - ChIP Grade (ab176880)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling Histone H3 (mono methyl K9) with ab176880 at 1/2000 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/400 dilution (green). Nuclear staining on HeLa cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows;

1. ab176880 at 1/2000 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.

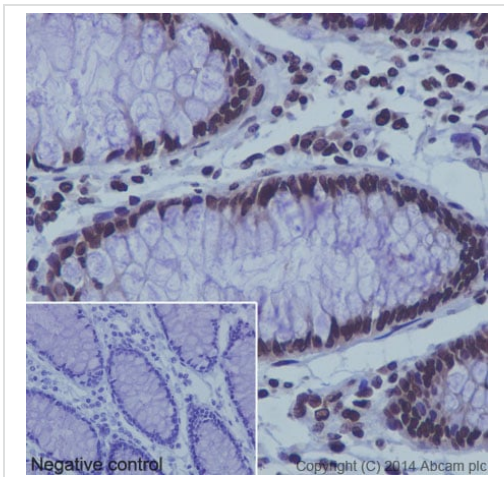


Peptide Array - Anti-Histone H3 (mono methyl K9) antibody [EPR16989] - ChIP Grade (ab176880)

ab176880 was tested in Peptide Array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate).

Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.

The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded [here](#).

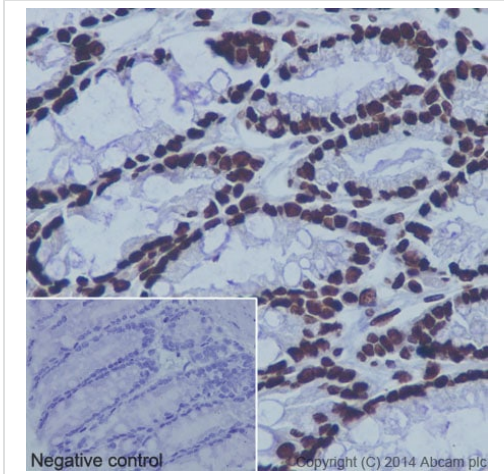


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mono methyl K9) antibody [EPR16989] - ChIP Grade (ab176880)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H3 (mono methyl K9) with ab176880 at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of colon tissue is observed. Counter stained with Hematoxylin.

Negative control: PBS instead of primary ab, secondary ab is prediluted Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

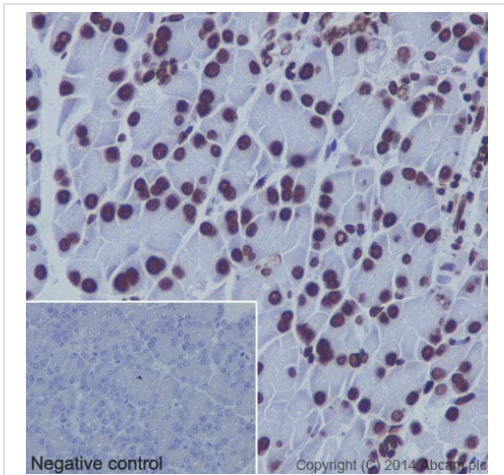


Immunohistochemical analysis of paraffin-embedded Mouse colon tissue labeling Histone H3 (mono methyl K9) with ab176880 at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of colon tissue is observed. Counter stained with Hematoxylin.

Negative control: PBS instead of primary ab, secondary ab is prediluted Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mono methyl K9) antibody [EPR16989] - ChIP Grade (ab176880)



Immunohistochemical analysis of paraffin-embedded rat pancreas tissue labeling Histone H3 (mono methyl K9) with ab176880 at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of pancreas tissue is observed. Counter stained with Hematoxylin.

Negative control: PBS instead of primary ab, secondary ab is prediluted Goat Anti-Rabbit IgG H&L (HRP).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mono methyl K9) antibody [EPR16989] - ChIP Grade (ab176880)

Why choose a recombinant antibody?



Research with confidence
Consistent and reproducible results



Long-term and scalable supply
Recombinant technology



Success from the first experiment
Confirmed specificity



Ethical standards compliant
Animal-free production

Anti-Histone H3 (mono methyl K9) antibody
[EPR16989] - ChIP Grade (ab176880)

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

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