

Product datasheet

Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade ab176877

Recombinant RabMAb

★★★★☆ 2 Abreviews 4 References 7 Images

Overview

Product name	Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade
Description	Rabbit monoclonal [ERP16597] to Histone H3 (mono methyl K4) - ChIP Grade
Host species	Rabbit
Tested applications	Suitable for: PepArr, WB, ICC/IF, IHC-P, ChIP
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Histone H3 aa 1-100 (mono methyl K4). The exact sequence is proprietary. Database link: P68431
Positive control	WB: HeLa and NIH/3T3 whole cell lysates. IHC-P: Human, mouse and rat colon tissues. ICC/IF: HeLa cells. ChIP: Chromatin from HeLa cells.

General notes

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMab[®] patents](#).

This product is a [recombinant rabbit monoclonal antibody](#).

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: PBS, 0.05% BSA, 40% Glycerol
Purity	Protein A purified
Clonality	Monoclonal
Clone number	ERP16597
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab176877** 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.
WB	★★★★☆	1/5000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ICC/IF		1/1000.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ChIP		Use at an assay dependent concentration. Use 2 µl.

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

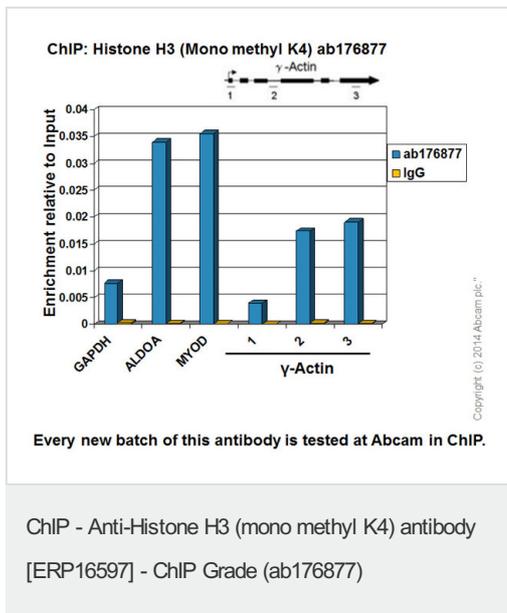
Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin. Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. 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.

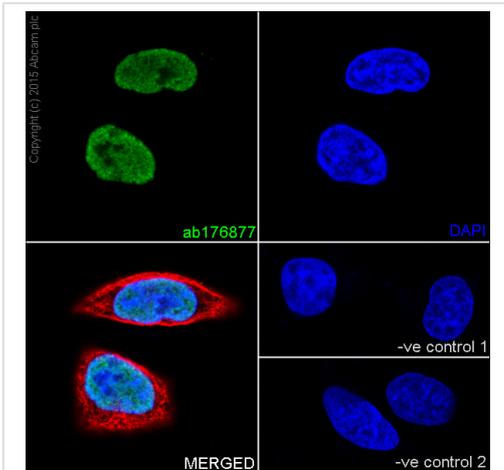
Cellular localization

Nucleus. Chromosome.

Images



Chromatin was prepared from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol. Cells were fixed with 0.75% formaldehyde for 10min. The ChIP was performed with 25 μ g of chromatin, 2 μ g of ab176877 (blue), and 20 μ l of Anti-rabbit IgG agarose beads. Rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified on the GAPDH and ALDOA (active) and MYO-D (inactive) promoters and over the γ -Actin gene (active). Schematic diagram of the γ -Actin gene is shown on the top of the figure. Black boxes represent exons and thin lines represent introns. PCR products are depicted as bars under the gene.

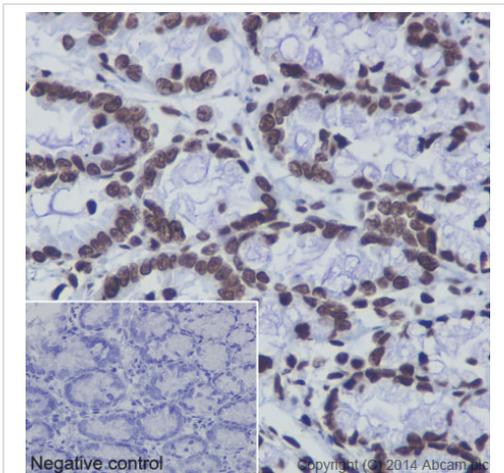


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade (ab176877)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Histone H3 (mono methyl K4) with ab176877 at 1/1000 dilution, followed by goat anti-rabbit Alexa Fluor[®] 488 (IgG) (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 (Alexa Fluor[®] 594 goat anti-mouse secondary) at 1/500 dilution (red).

The negative controls are as follows;

1. ab176877 at 1/1000 dilution followed by ab150120 (goat anti-mouse Alexa Fluor[®] 594 secondary) at 1/500 dilution.
2. ab7291 (anti-Tubulin mouse mAb) at 1/500 dilution followed by ab150077 (goat anti-rabbit Alexa Fluor[®] 488; IgG H&L) at 1/400 dilution.

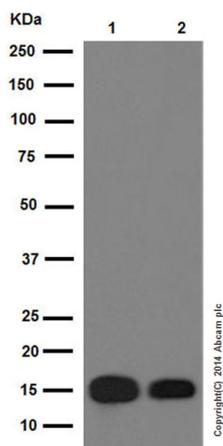


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade (ab176877)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling Histone H3 (mono methyl K4) with ab176877 at 1/1000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of colon tissue is observed. Counterstained 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.



Western blot - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade (ab176877)

All lanes : Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade (ab176877) at 1/5000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysates

Lane 2 : NIH/3T3 (Mouse embryo fibroblast cell line) whole cell lysates

Lysates/proteins at 10 µg per lane.

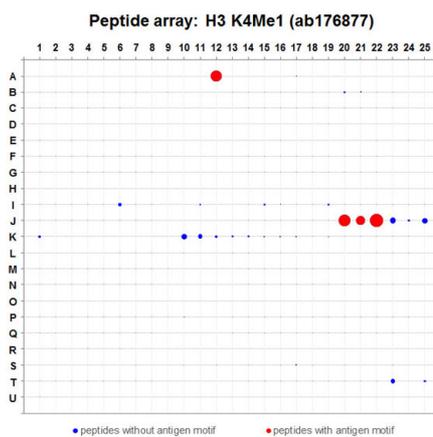
Secondary

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.

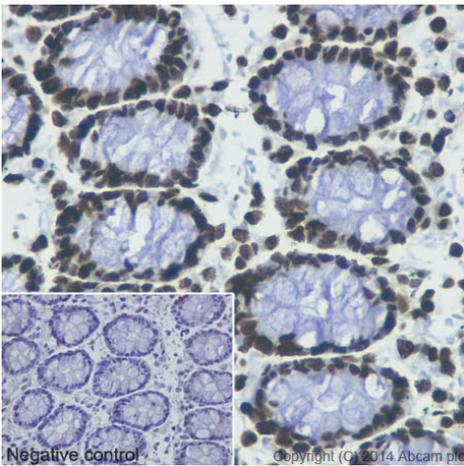


Peptide Array - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade (ab176877)

ab176877 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](#).

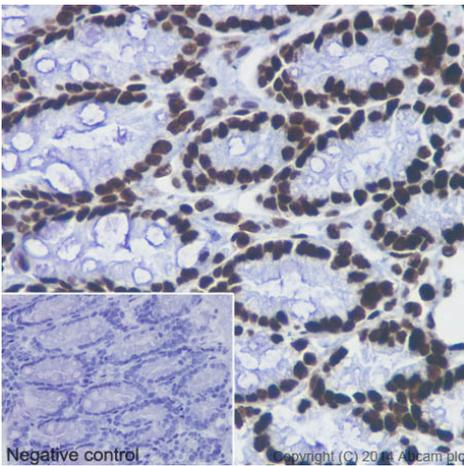


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade (ab176877)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Histone H3 (mono methyl K4) with ab176877 at 1/1000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of colon tissue is observed. Counterstained 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 K4) antibody [ERP16597] - ChIP Grade (ab176877)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Histone H3 (mono methyl K4) with ab176877 at 1/1000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of colon tissue is observed. Counterstained 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.

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