

Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade - BSA and Azide free ab239402

Recombinant RabMAb

8 Images

Overview

Product name	Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade - BSA and Azide free
Description	Rabbit monoclonal [ERP16597] to Histone H3 (mono methyl K4) - ChIP Grade – BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: ChIP-sequencing, WB, IHC-P, ICC/IF, ChIP, PepArr, ChIC/CUT&RUN-seq
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human, mouse and rat colon tissues. ICC/IF: HeLa cells. ChIP: Chromatin from HeLa cells. ChIC/CUT&RUN-Seq: HeLa cells.
General notes	ab239402 is the carrier-free version of ab176877 .

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	ERP16597
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab239402 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
ChIP-sequencing		Use at an assay dependent concentration.
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. 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.
ChIP		Use at an assay dependent concentration.
PepArr		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		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 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.

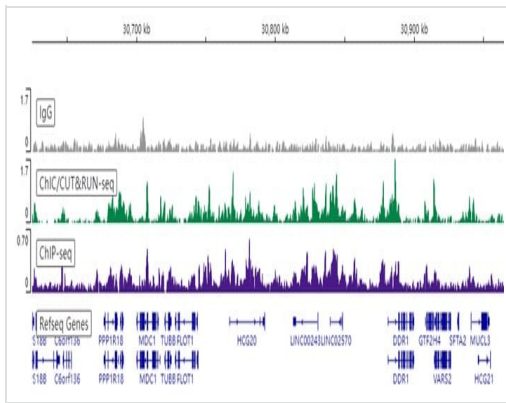
Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin.

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



ChIC/CUT&RUN sequencing - Anti-Histone H3
(mono methyl K4) antibody [ERP16597] - ChIP
Grade - BSA and Azide free (ab239402)

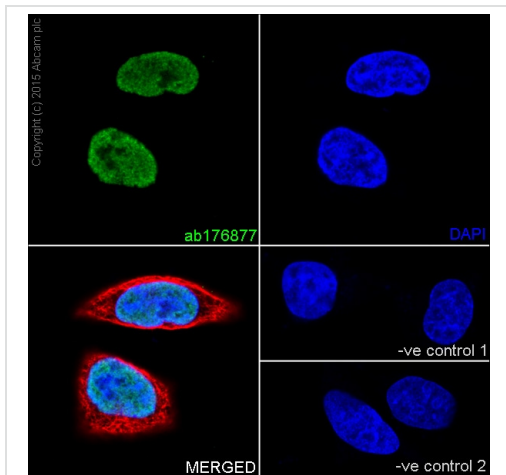
ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5×10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 2 μ g of **ab176877** [ERP16597]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

The ChIP data was conducted on chromatin prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 4 μ g of **ab176877**. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded [here](#).

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab176877**).



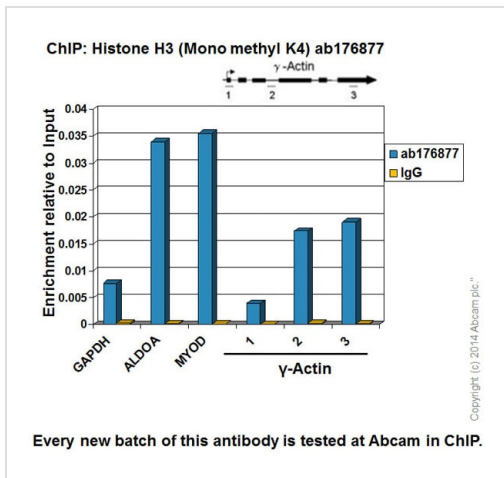
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade - BSA and Azide free (ab239402)

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.

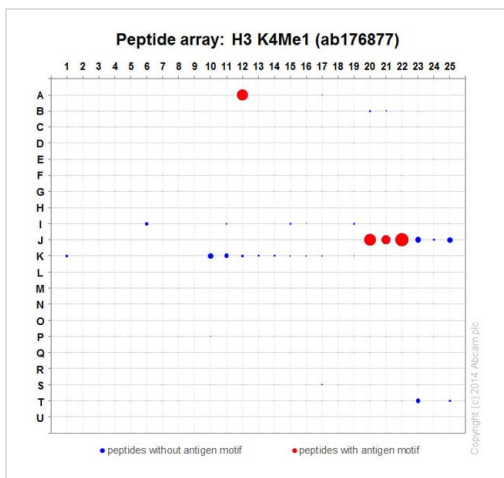
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab176877**).



ChIP - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade - BSA and Azide free (ab239402)

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 25µg of chromatin, 2µg of **ab176877** (blue), and 20µl 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab176877**).



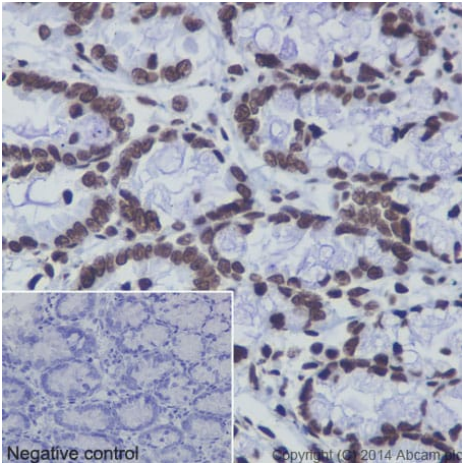
Peptide Array - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade - BSA and Azide free (ab239402)

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](#).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab176877**).



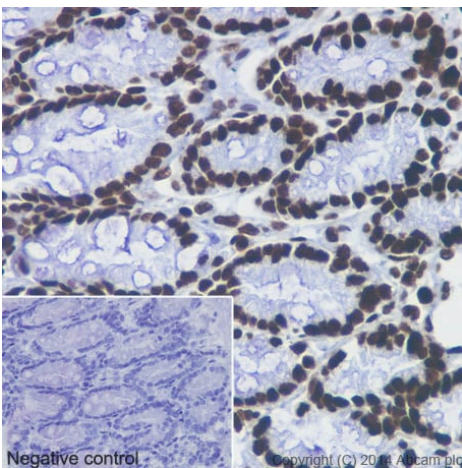
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - CHIP Grade - BSA and Azide free (ab239402)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab176877](#)).

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



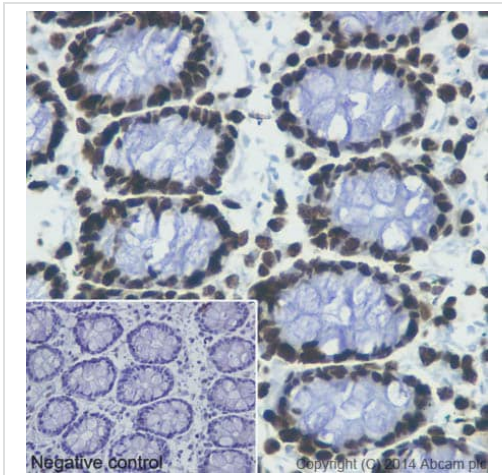
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (mono methyl K4) antibody [ERP16597] - CHIP Grade - BSA and Azide free (ab239402)

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. Counter stained with hematoxylin.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab176877](#)).

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



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. Counter stained with hematoxylin.





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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab176877](#)).

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 - BSA and Azide free (ab239402)

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-Histone H3 (mono methyl K4) antibody [ERP16597] - ChIP Grade - BSA and Azide free (ab239402)

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

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