abcam

Product datasheet

Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade ab177177

リコンビナント RabMAb

1 Abreviews 4 References 画像数 13

製品の概要

製品名	Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR16988] to Histone H3 (acetyl K9) - ChIP Grade
由来種	Rabbit
アプリケーション	**適用あり:** ICC/IF, IHC-P, WB, PepArr, ChIP-sequencing, ChIC/CUT&RUN-seq, ChIP
種交差性	**交差種:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Whole cell lysates from HeLa and NIH/3T3 cells treated with Trichostatin A. IHC-P: Human and mouse colon tissue. Rat spleen tissue. ICC/IF: HeLa cells. ChIP-seq: Chromatin from HeLa cells. ChIC/CUT&RUN: HeLa cells.
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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 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	EPR16988

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ab177177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
ICC/IF		1/3000.
IHC-P		1/200. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★☆☆ (1)	1/5000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
PepArr		Use at an assay dependent concentration.
ChIP-sequencing		Use 4µg for 10^7 cells.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5 µg
ChIP		Use 2 µg for 25 µg of chromatin.

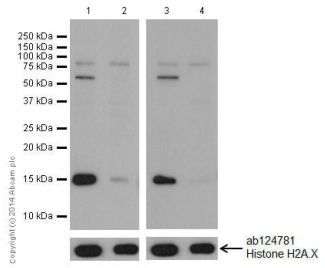
ターゲット情報

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



Western blot - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

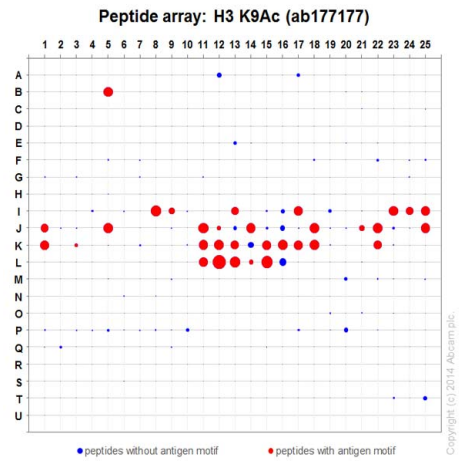
All lanes : Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177) at 1/1000 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with Trichostatin A 500 ng/ml for 4hr whole cell lysates
Lane 2 : Untreated HeLa whole cell lysates
Lane 3 : NIH/3T3 (Mouse embryo fibroblast cell line) treated with Trichostatin A 500 ng/ml for 4hr whole cell lysates
Lane 4 : Untreated NIH/3T3 whole cell lysates

Lysates/proteins at 10 µg per lane.

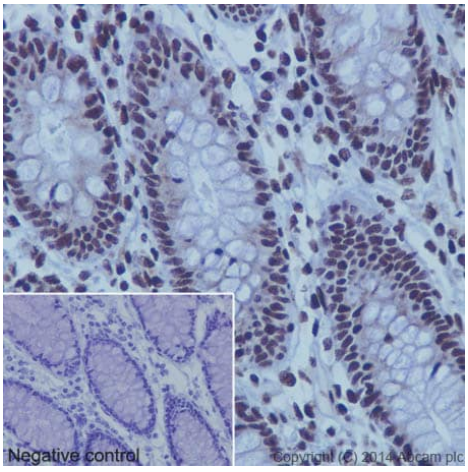
Predicted band size: 15 kDa

Observed band size: 15 kDa



Peptide Array - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

ab177177 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**.

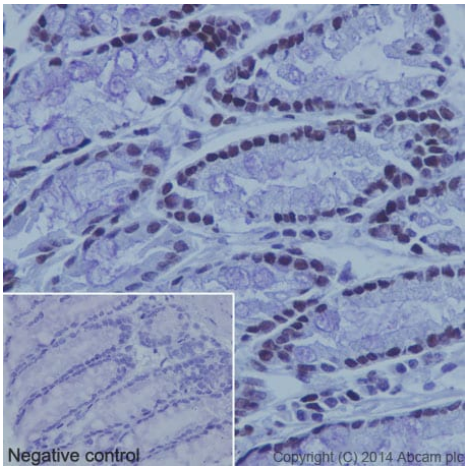


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Histone H3 (acetyl K9) with ab177177 at 1/500 dilution, followed by Goat Anti-Rabbit HRP (IgG H&L) (**ab97051**) at 1/500 dilution. Nuclear staining on glandular epithelium of Human colon tissue is observed. Counterstained with hematoxylin.

Negative control: PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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

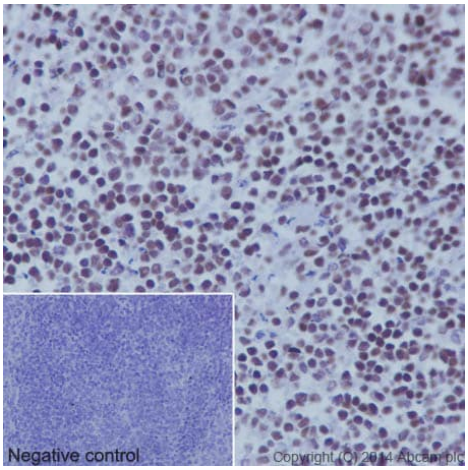


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Histone H3 (acetyl K9) with ab177177 at 1/500 dilution, followed by Goat Anti-Rabbit HRP (IgG H&L) (**ab97051**) at 1/500 dilution. Nuclear staining on glandular epithelium of mouse colon tissue is observed. Counterstained with hematoxylin.

Negative control: PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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

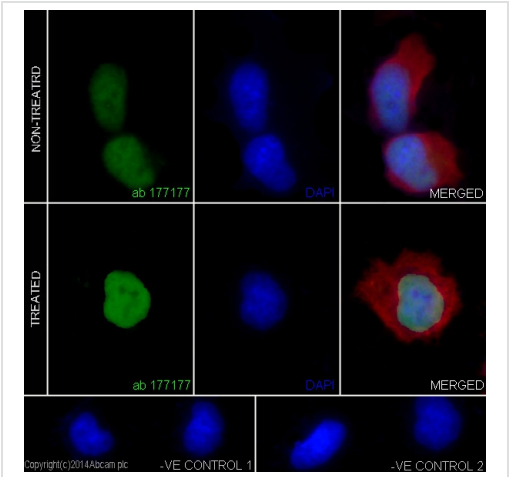


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling Histone H3 (acetyl K9) with ab177177 at 1/500 dilution, followed by Goat Anti-Rabbit HRP (IgG H&L) (**ab97051**) at 1/500 dilution. Nuclear staining on rat spleen tissue is observed. Counterstained with hematoxylin.

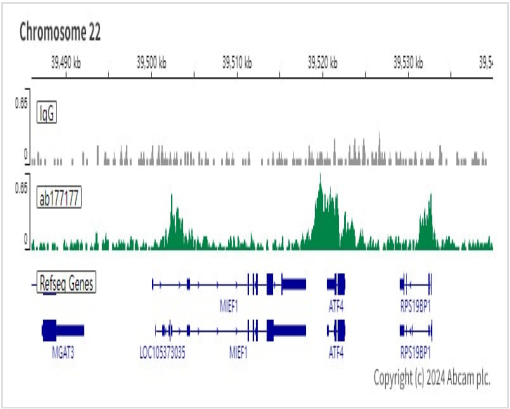
Negative control: PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

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



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

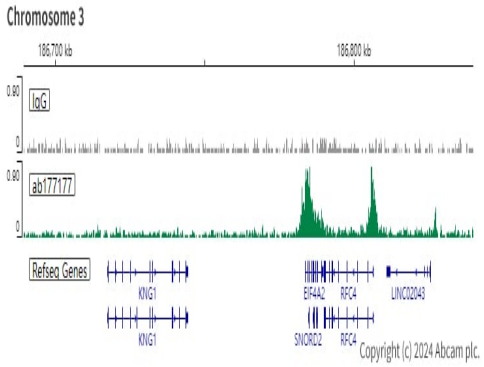
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Histone H3 (acetyl K9) with ab177177 at 1/3000 dilution, followed by Goat anti-rabbit Alexa Fluor® 488 (IgG) (**ab150077**) secondary antibody at 1/400 dilution (green). Confocal image shows nuclear staining on HeLa cell line. The expression increased after treatment with Trichostatin A (500 ng/ml) for 4 hours. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (goat anti-mouse AlexaFluor®594 secondary antibody) at 1/500 dilution (red).
The negative controls are as follows:
-ve control 1: ab177177 at 1/3000 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
-ve control 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.



ChIC/CUT&RUN sequencing - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

ChIC/CUT&RUN was performed using a pAG-MNase at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells and 5 µg of ab177177 [EPR16988]. 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 University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



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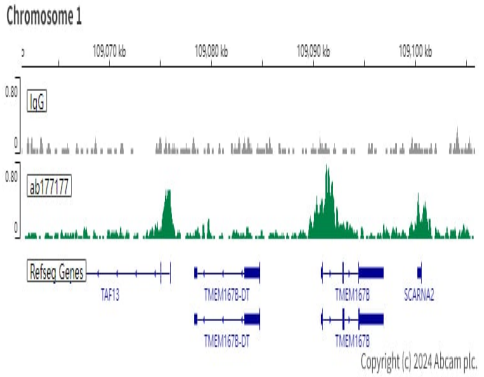

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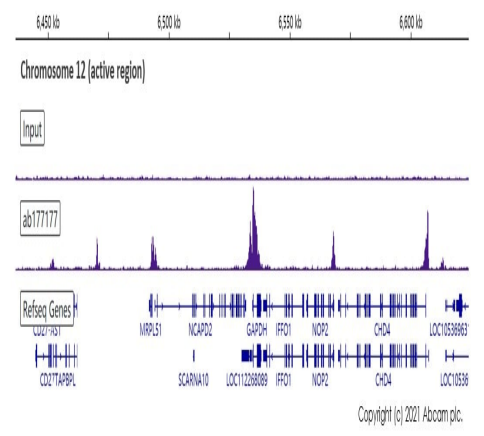
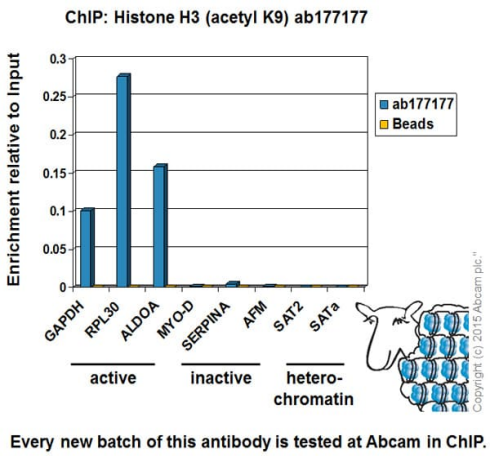


ChIP-sequencing - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 4 µg of ab177177 [EPR16988]. 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**.



ChIP - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

Chromatin was prepared from HeLa (Human epithelial cell line from cervix adenocarcinoma) 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 ab177177 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). 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.

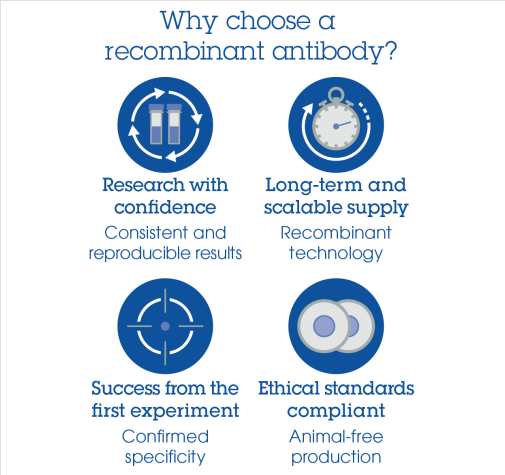
CUT&Tag sequencing - Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

This experiment and image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet.

CUT&Tag-seq was performed using 200,000 Oli-neu (Oligodendrocyte progenitor) cells. Cells were permeabilized with 0.05% Digitonin and 0.01% NP-40 for 3 minutes. A 1:100 dilution of Recombinant Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177) was used, along with a Guinea pig anti-rabbit Secondary. DNA was seg using Illumina NovaSeq S Prime to a depth of 22 million reads.

This image is courtesy of Dr Marek Bartosovic, Gonçalo Castelo-Branco Group, Karolinska Institutet.

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



Anti-Histone H3 (acetyl K9) antibody [EPR16988] - ChIP Grade (ab177177)

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