abcam

Product datasheet

Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade ab177188

Recombinant RabMAb

3 Abreviews 5 References 9 Images

Overview

Product name	Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade
Description	Rabbit monoclonal [EPR16999] to Histone H4 (mono methyl K20) - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** ChIP-sequencing, PepArr, ICC/IF, ChIP, IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	ICC/IF: HeLa cells. IHC-P: Human and mouse colon tissues; Rat kidney tissue. ChIP: Chromatin from HeLa cells. WB: HeLa whole cell lysates; NIH3T3 nuclear extracts lysates. ChIP-seq: Chromatin prepared from Hela cells.
General notes	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 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, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR16999

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab177188 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 4µg for 10^7 cells.
PepArr		Use at an assay dependent concentration.
ICC/IF		1/100.
ChIP	★★★★★ (2)	Use 2 µg for 25 µg of chromatin.
IHC-P		1/100. 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 11 kDa (predicted molecular weight: 11 kDa).

Target

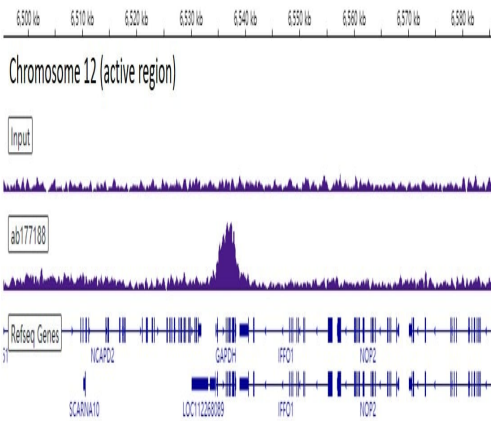
Function Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA 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 H4 family.

Post-translational modifications Acetylation at Lys-6 (H4K5ac), Lys-9 (H4K8ac), Lys-13 (H4K12ac) and Lys-17 (H4K16ac) occurs in coding regions of the genome but not in heterochromatin.
Citrullination at Arg-4 (H4R3ci) by PADI4 impairs methylation.
Monomethylation and asymmetric dimethylation at Arg-4 (H4R3me1 and H4R3me2a, respectively) by PRMT1 favors acetylation at Lys-9 (H4K8ac) and Lys-13 (H4K12ac).
Demethylation is performed by JMJD6. Symmetric dimethylation on Arg-4 (H4R3me2s) by the PRDM1/PRMT5 complex may play a crucial role in the germ-cell lineage.
Monomethylated, dimethylated or trimethylated at Lys-21 (H4K20me1, H4K20me2, H4K20me3). Monomethylation is performed by SET8. Trimethylation is performed by SUV420H1 and SUV420H2 and induces gene silencing.
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. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage. The exact role of H4K91ub1 in DNA damage response is still unclear but it may function as a licensing signal for additional histone H4 post-translational modifications such as H4 Lys-21 methylation (H4K20me).
Sumoylated, which is associated with transcriptional repression.

Cellular localization Nucleus. Chromosome.

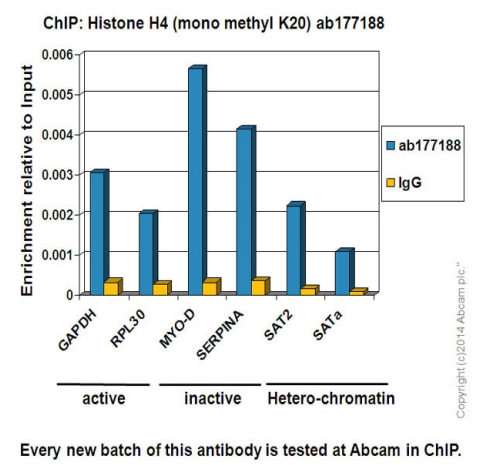
Images



ChIP-sequencing - Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

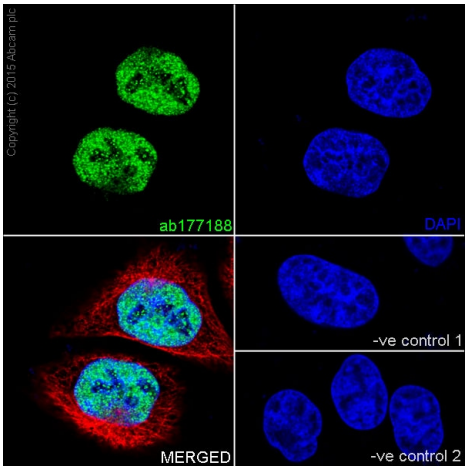
Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with 10^7 HeLa cells and 4 µg of ab177188 [EPR16999]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.



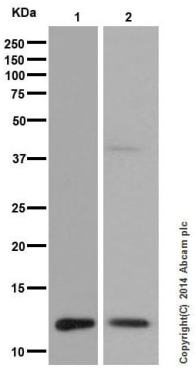
ChIP - Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 2µg of ab177188 (blue), and 20µl of Anti rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (SYBR approach). Primers are located in the first kb of the transcribed region.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling Histone H4 (mono methyl K20) with ab177188 at 1/100 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. ab177188 at 1/100 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.



Western blot - Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

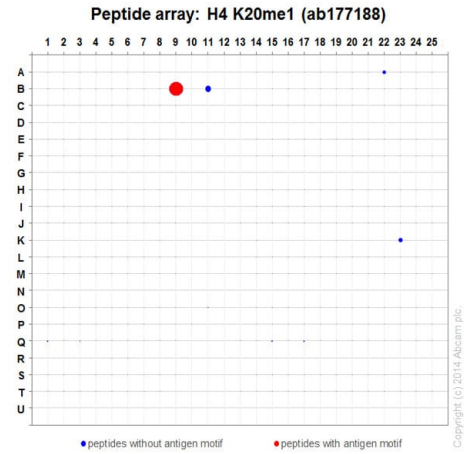
All lanes : Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188) at 1/5000 dilution

Lane 1 : Hela whole cell lysates
Lane 2 : NIH/3T3 whole cell lysates

Lysates/proteins at 10 µg per lane.

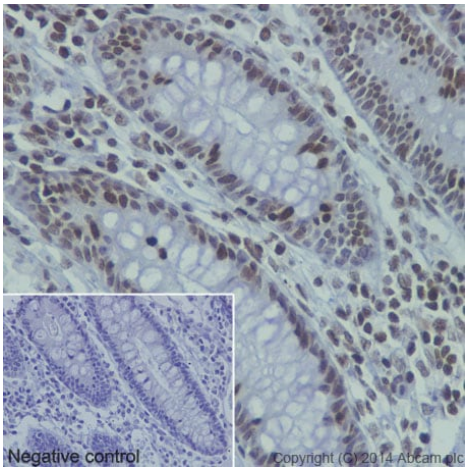
Predicted band size: 11 kDa
Observed band size: 11 kDa

Blocking buffer/Diluting buffer: 5% NFDM/TBST



Peptide Array - Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

ab177188 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**.

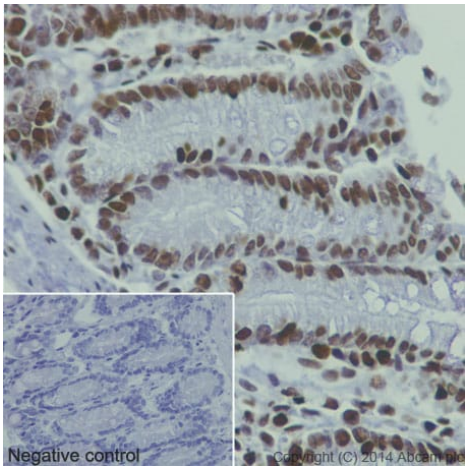


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H4 (mono methyl K20) with ab177188 at 1/100 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 antibody, secondary antibody 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.

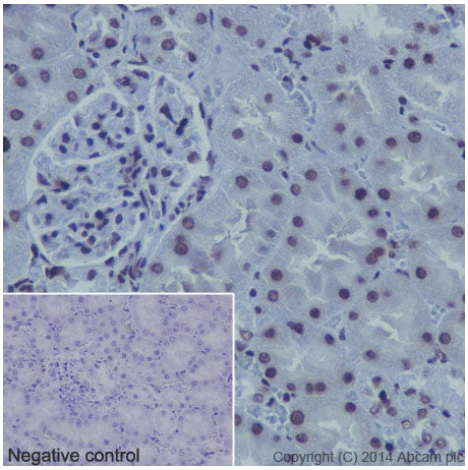


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Histone H4 (mono methyl K20) with ab177188 at 1/100 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of mouse colon tissue is observed. Counter stained with Hematoxylin.

Negative control: PBS instead of primary antibody, secondary antibody 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.

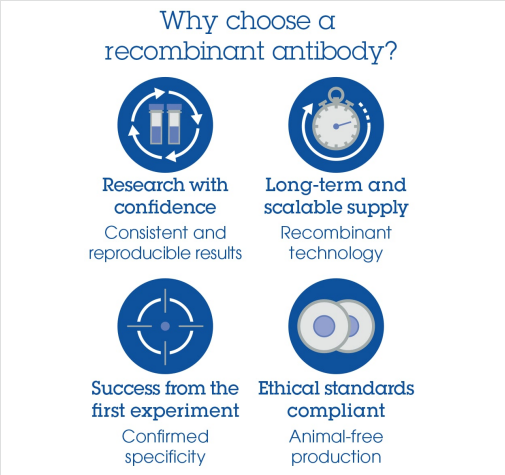


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling Histone H4 (mono methyl K20) with ab177188 at 1/100 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on rat kidney tissue is observed. Counter stained with Hematoxylin.

Negative control: PBS instead of primary antibody, secondary antibody 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.



Anti-Histone H4 (mono methyl K20) antibody [EPR16999] - ChIP Grade (ab177188)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors