


## Product datasheet

# Anti-Histone H4 antibody [EPR16599] - ChIP Grade ab177840

Recombinant RabMAb

★★★★☆ [4 Abreviews](#) [16 References](#) [10 Images](#)

### Overview

<b>Product name</b>	Anti-Histone H4 antibody [EPR16599] - ChIP Grade
<b>Description</b>	Rabbit monoclonal [EPR16599] to Histone H4 - ChIP Grade
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> PepArr, ChIP, IHC-P, WB, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human, Drosophila melanogaster, Recombinant fragment <b>Predicted to work with:</b> a wide range of other species 
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	WB: HeLa and NIH/3T3 whole cell lysates; Drosophila whole lysate. ICC/IF: HeLa cells. IHC-P: Human colon, mouse pancreas and rat cerebral cortex tissues. ChIP: Chromatin from HeLa cells.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAb<sup>®</sup> patents</a>.</p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR16599

Isotype

IgG

## Applications

### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab177840 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.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ (4)	1/1000. Detects a band of approximately 11 kDa (predicted molecular weight: 11 kDa). We recommend using 3% milk as the blocking agent for Western blot.
ICC/IF		1/100.

## 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.

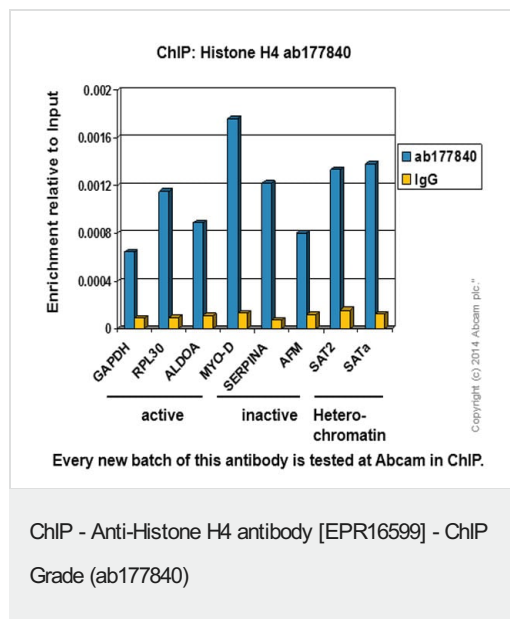
### Sequence similarities

Belongs to the histone H4 family.

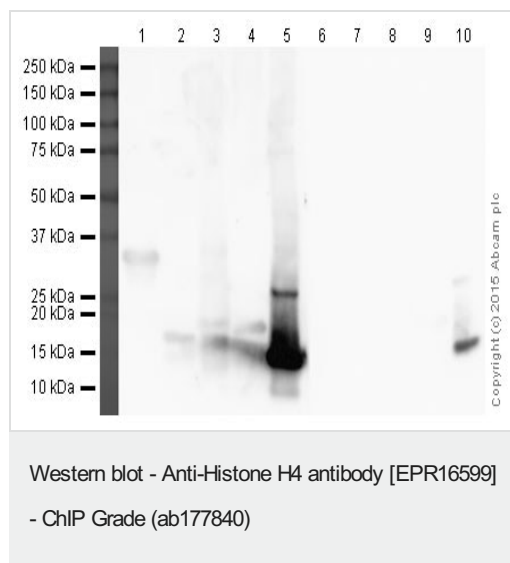
### Post-translational modifications

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.  
Citruination at Arg-4 (H4R3ci) by PAD4 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.

## Images



Chromatin was prepared from Hela 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 ab177840 (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.



**All lanes :** Anti-Histone H4 antibody [EPR16599] - ChIP Grade (ab177840) at 1/1000 dilution

**Lanes 1 & 6 :** Histone H1 Recombinant Protein

**Lanes 2 & 7 :** Histone H2A Recombinant Protein

**Lanes 3 & 8 :** Histone H2B Recombinant Protein

**Lanes 4 & 9 :** Histone H3.1 Recombinant Protein

**Lanes 5 & 10 :** Histone H4 Recombinant Protein

Lysates/proteins at 0.1 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG (H+L), Peroxidase Conjugated at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 11 kDa

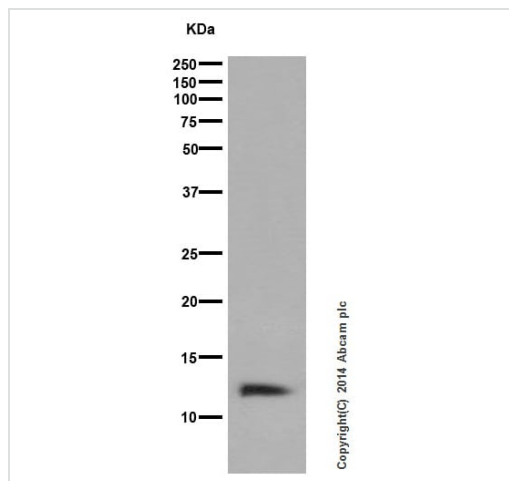
**Observed band size:** 15 kDa

**Exposure time:** 4 minutes

Lanes 1-5: 1% BSA blocking buffer

Lanes 6-10: 3% Milk blocking buffer

We recommend using 3% milk as the blocking agent for Western blot.



Western blot - Anti-Histone H4 antibody [EPR16599]  
- ChIP Grade (ab177840)

Anti-Histone H4 antibody [EPR16599] - ChIP Grade (ab177840) at 1/1000 dilution + Drosophila whole lysates at 10 µg

### Secondary

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

Developed using the ECL technique.

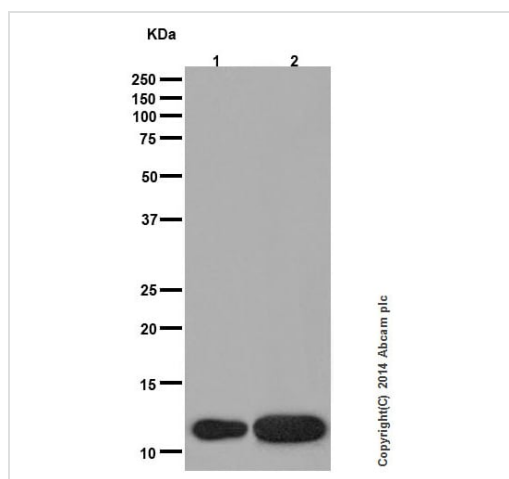
Performed under reducing conditions.

**Predicted band size:** 11 kDa

**Observed band size:** 11 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFD/MTBST.



Western blot - Anti-Histone H4 antibody [EPR16599]  
- ChIP Grade (ab177840)

**All lanes :** Anti-Histone H4 antibody [EPR16599] - ChIP Grade (ab177840) at 1/5000 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

Developed using the ECL technique.

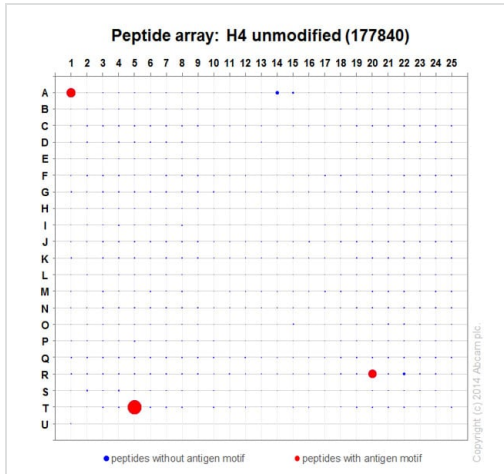
Performed under reducing conditions.

**Predicted band size:** 11 kDa

**Observed band size:** 11 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

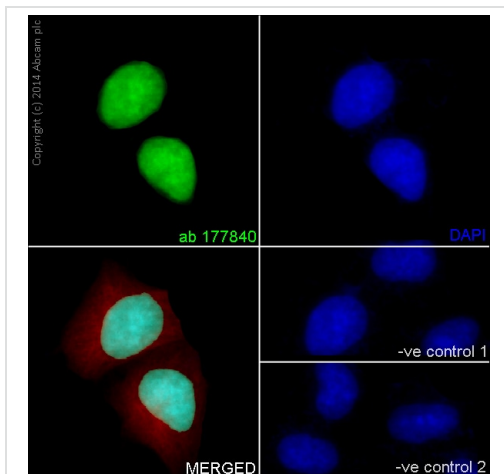


Peptide Array - Anti-Histone H4 antibody  
[EPR16599] - ChIP Grade (ab177840)

ab177840 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](#).

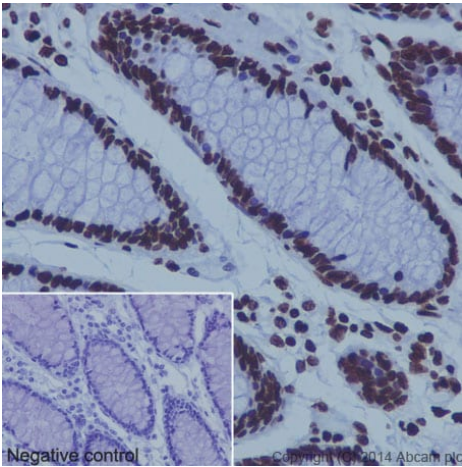


Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 antibody [EPR16599] - ChIP Grade (ab177840)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling Histone H4 with ab177840 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. ab177840 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.

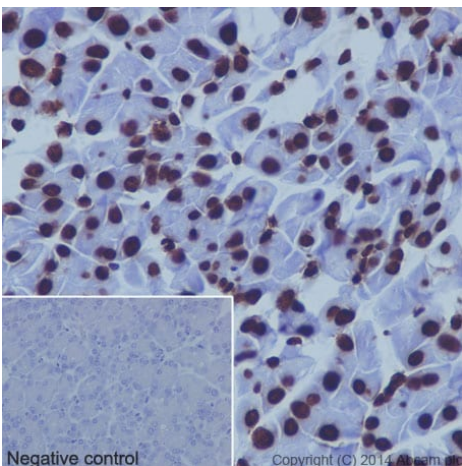


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 antibody [EPR16599] - ChIP Grade (ab177840)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H4 with ab177840 at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of Human 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) ([ab97051](#)) at 1/500 dilution.

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



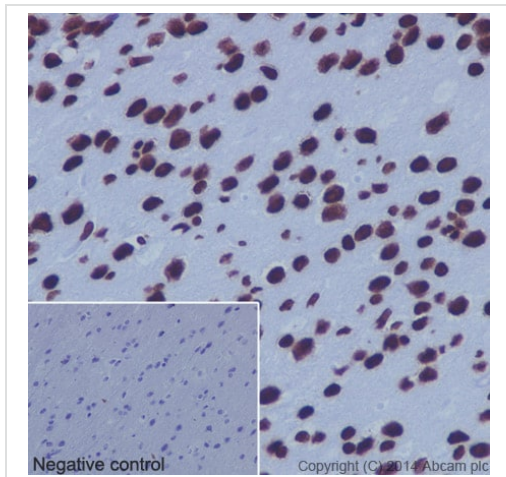
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 antibody [EPR16599] - ChIP Grade (ab177840)

Immunohistochemical analysis of paraffin-embedded Mouse pancreas tissue labeling Histone H4 with ab177840 at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nucleus staining on glandular epithelium of mouse pancreas 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) ([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 H4 antibody [EPR16599] - ChIP Grade (ab177840)

Immunohistochemical analysis of paraffin-embedded Rat cerebral cortex tissue labeling Histone H4 with ab177840 at 1/2000 dilution, followed by prediluted Goat Anti-Rabbit IgG H&L (HRP). Nuclear staining on neuron cells of cerebral cortex 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) ([ab97051](#)) at 1/500 dilution.

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

Why choose a recombinant antibody?

 <p><b>Research with confidence</b> Consistent and reproducible results</p>	 <p><b>Long-term and scalable supply</b> Recombinant technology</p>
 <p><b>Success from the first experiment</b> Confirmed specificity</p>	 <p><b>Ethical standards compliant</b> Animal-free production</p>

Anti-Histone H4 antibody [EPR16599] - ChIP Grade (ab177840)

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