

Product datasheet

# Anti-Histone H3 (acetyl K64) antibody [EPR20713] - ChIP Grade ab214808

Recombinant RabMAb

5 Images

Overview

<b>Product name</b>	Anti-Histone H3 (acetyl K64) antibody [EPR20713] - ChIP Grade
<b>Description</b>	Rabbit monoclonal [EPR20713] to Histone H3 (acetyl K64) - ChIP Grade
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> PepArr, ChIP, WB, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human <b>Predicted to work with:</b> Goat, Arabidopsis thaliana, Drosophila melanogaster, Pufferfish, Non human primates, Zebrafish, Xenopus tropicalis, Camel, Northern treeshrew, Nine-banded armadillo 
<b>Immunogen</b>	Synthetic peptide within Human Histone H3 aa 50 to the C-terminus (acetyl K64). The exact sequence is proprietary. Database link: <a href="#">P68431</a>
<b>Positive control</b>	WB: NIH/3T3 and C6 treated with 500ng/ml Trichostatin A for 4 hours whole cell lysates; HeLa treated with 400ng/ml Trichostatin A for 9 hours whole cell lysate. ICC/IF: HeLa cells. ChIP: Chromatin prepared from HeLa cells.
<b>General notes</b>	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> .  This product is a <a href="#">recombinant rabbit monoclonal antibody</a> .

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: 0.05% BSA, 40% Glycerol, PBS
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR20713

Isotype

IgG

## Applications

Our [Abpromise guarantee](#) covers the use of **ab214808** 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 a concentration of 0.1 µg/ml.
ChIP		Use 2 µg for 25 µg of chromatin.
WB		1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ICC/IF		1/2000.

## 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

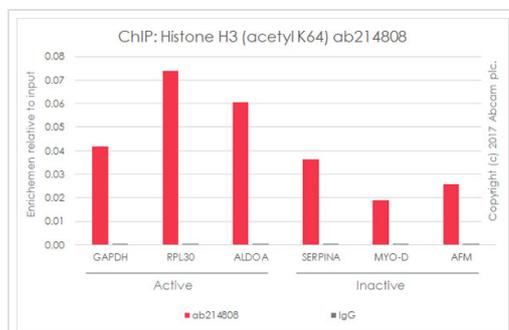
Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me). Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PAD4 impairs methylation 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

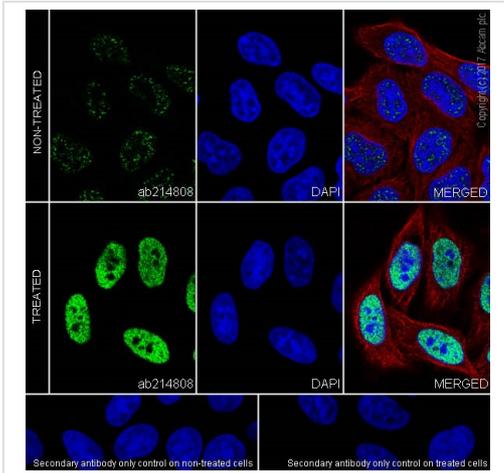
Nucleus. Chromosome.

## Images



ChIP - Anti-Histone H3 (acetyl K64) antibody  
[EPR20713] - ChIP Grade (ab214808)

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 ab214808(red), and 20µl of Anti rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region.

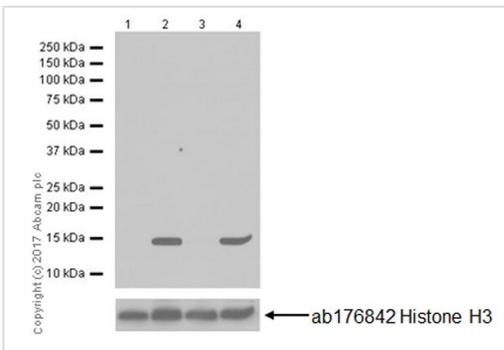


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K64) antibody [EPR20713] - ChIP Grade (ab214808)

Immunofluorescent analysis of 100% methanol-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells, untreated and treated with Trichostatin A (500 ng/ml) for 4 hours, labeling Histone H3 (acetyl K64) with ab214808 at 1/2000 dilution followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cells. 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 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (ab195889) (red) at 1/200 dilution.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution.



Western blot - Anti-Histone H3 (acetyl K64) antibody [EPR20713] - ChIP Grade (ab214808)

**All lanes :** Anti-Histone H3 (acetyl K64) antibody [EPR20713] - ChIP Grade (ab214808) at 1/5000 dilution

**Lane 1 :** Untreated NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate

**Lane 2 :** NIH/3T3 treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate

**Lane 3 :** Untreated C6 (rat glial tumor cell line) whole cell lysate

**Lane 4 :** C6 treated with 500ng/ml Trichostatin A for 4 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

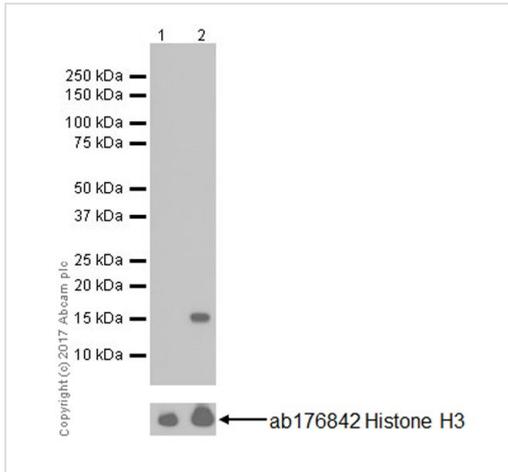
Developed using the ECL technique.

**Predicted band size:** 15 kDa

**Observed band size:** 15 kDa

**Exposure time:** 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Histone H3 (acetyl K64) antibody [EPR20713] - ChIP Grade (ab214808)

**All lanes :** Anti-Histone H3 (acetyl K64) antibody [EPR20713] - ChIP Grade (ab214808) at 1/1000 dilution

**Lane 1 :** Untreated HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

**Lane 2 :** HeLa treated with 400ng/ml Trichostatin A for 9 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

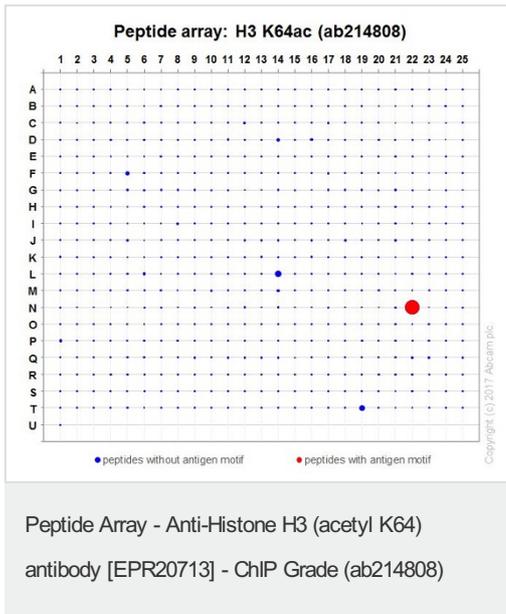
Developed using the ECL technique.

**Predicted band size:** 15 kDa

**Observed band size:** 15 kDa

**Exposure time:** 15 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



ab214808 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](#).

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

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