abcam

Product datasheet

Anti-Histone H3 (acetyl K64) antibody [EPR20713] - BSA and Azide free ab251549

Recombinant RabMAb

★★★★★ 1 Abreviews 7 Images

Overview

Product name	Anti-Histone H3 (acetyl K64) antibody [EPR20713] - BSA and Azide free
Description	Rabbit monoclonal [EPR20713] to Histone H3 (acetyl K64) - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** PepArr, ChIP, ChIP-sequencing, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Goat, Arabidopsis thaliana, Drosophila melanogaster, Pufferfish, Non human primates, Zebrafish, Xenopus tropicalis, Camel, Northern treeshrew, Nine-banded armadillo
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	ab251549 is the carrier-free version of **ab214808**. 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	EPR20713
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab251549 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 at an assay dependent concentration.
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).
ICC/IF		Use at an assay dependent concentration.

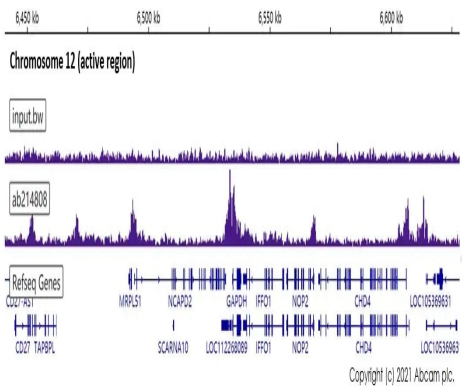
Target

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 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.

Cellular localization Nucleus. Chromosome.

Images

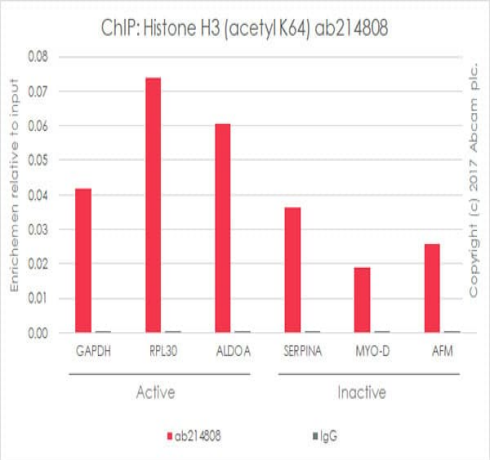


ChIP-sequencing - Anti-Histone H3 (acetyl K64) antibody [EPR20713] - BSA and Azide free (ab251549)

This data was developed using **ab214808**, the same antibody clone in a different buffer formulation.

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 **ab214808**. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

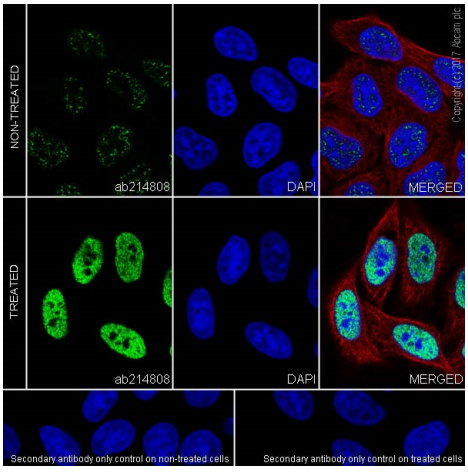
Additional screenshots of mapped reads can be downloaded **here**.



ChIP - Anti-Histone H3 (acetyl K64) antibody [EPR20713] - BSA and Azide free (ab251549)

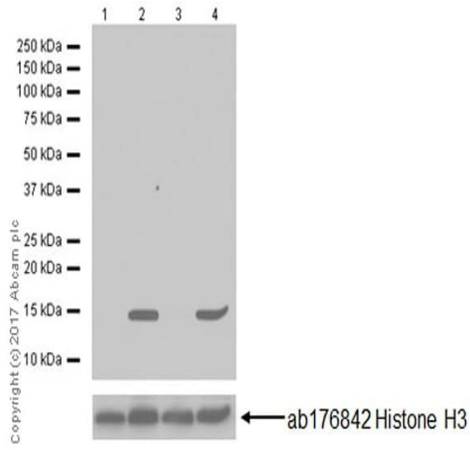
This data was developed using **ab214808**, the same antibody clone in a different buffer formulation.

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] - BSA and Azide free (ab251549)

This data was developed using **ab214808**, the same antibody clone in a different buffer formulation.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] - BSA and Azide free (ab251549)

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

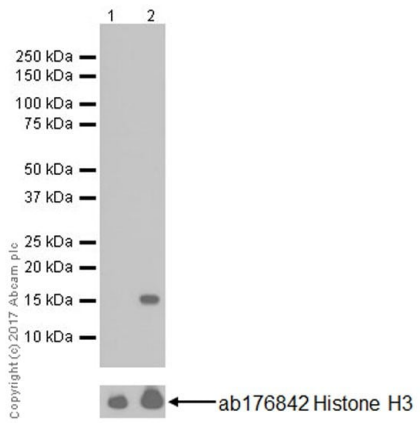
Developed using the ECL technique.

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 3 minutes

This data was developed using **ab214808**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Histone H3 (acetyl K64) antibody [EPR20713] - BSA and Azide free (ab251549)

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

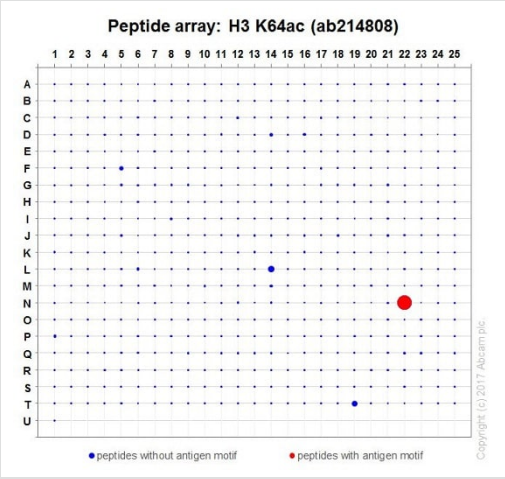
Developed using the ECL technique.

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 15 seconds

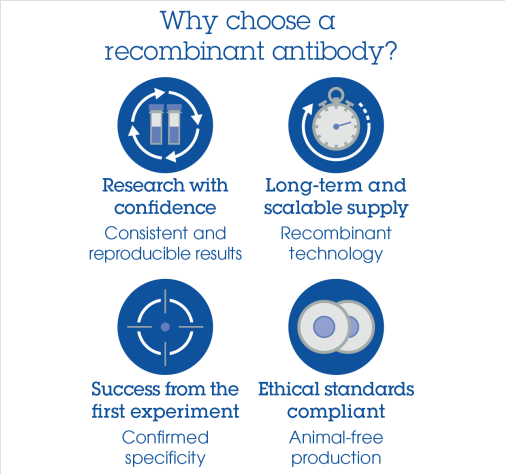
This data was developed using **ab214808**, the same antibody clone in a different buffer formulation.

Blocking and dilution buffer: 5% NFDM/TBST.



Peptide Array - Anti-Histone H3 (acetyl K64) antibody [EPR20713] - BSA and Azide free (ab251549)

This data was developed using **ab214808**, the same antibody clone in a different buffer formulation.**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**.



Anti-Histone H3 (acetyl K64) antibody [EPR20713] - BSA and Azide free (ab251549)

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