abcam

Product datasheet

Anti-Histone H3 (acetyl K14) antibody [EP964Y] - BSA and Azide free ab203952

Recombinant RabMAb

12 References 5 Images

Overview

Product name	Anti-Histone H3 (acetyl K14) antibody [EP964Y] - BSA and Azide free
Description	Rabbit monoclonal [EP964Y] to Histone H3 (acetyl K14) - BSA and Azide free
Host species	Rabbit
Specificity	There was no cross-reactivity observed with recombinant H3 or the following modifications Acetyl-K9/pS10, -K18, -K23, and -K27 in dot plot.
Tested applications	**Suitable for:** ICC/IF, ChIP, IHC-P, WB **Unsuitable for:** Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Saccharomyces cerevisiae, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: C6, Hek293T and HEL cell lysates. IHC-P: Human uterus adenocarcinoma and endometrium carcinoma tissues, Mouse kidney tissue and Rat liver tissue. ICC/IF: HeLa cells treated/untreated with trichostatin A. ChIP: Chromatin was prepared from HeLa cells (treated with 50 ng/ml nocodazole for 14 hours).
General notes	ab203952 is the carrier-free version of **ab52946**. 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. 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: 7.20 Constituent: 100% PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP964Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab203952 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
ICC/IF		Use at an assay dependent concentration.
ChIP		Use 5 µg for 25 µg of chromatin.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration. Detects a band of approximately 11 kDa (predicted molecular weight: 11 kDa).

Application notes Is unsuitable for Flow Cyt.

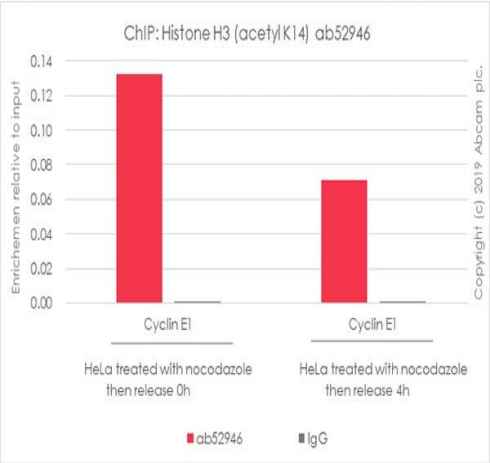
Target

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.

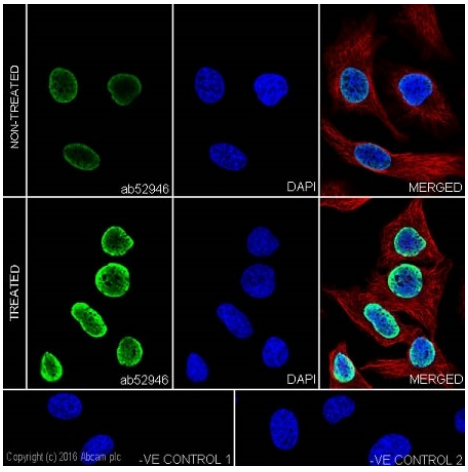
Images



ChIP - Anti-Histone H3 (acetyl K14) antibody [EP964Y] - BSA and Azide free (ab203952)

This data was developed using the same antibody clone in a different buffer formulation (**ab52946**).

Chromatin was prepared from HeLa cells (treated with 50 ng/ml nocodazole for 14 hours) according to the Abcam X-ChIP protocol. Cells were fixed with EGS (1.5 mM) for 30 minutes then formaldehyde (1%) for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of **ab52946** (red), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (TaqMan approach).



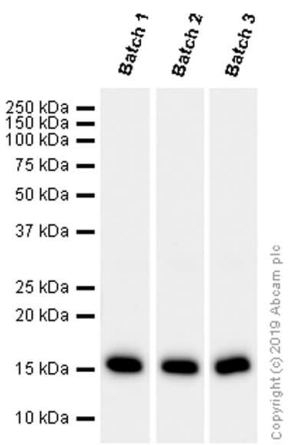
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K14) antibody [EP964Y] - BSA and Azide free (ab203952)

Immunocytochemistry/Immunofluorescence analysis of untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) cells and TSA (Trichostatin A) (500ng/ml, 4h) and treated HeLa cells labeling Histone H3 (acetyl K14) with purified **ab52946** at 1/500. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100, counterstained with **ab150120** AlexaFluor®594 Goat anti-Mouse secondary 1:1000 (2ug/ml). An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody (Ab150077). Nuclei counterstained with DAPI (blue).

Negative Control 1: Rabbit primary antibody and anti-mouse secondary antibody(**ab150120**)

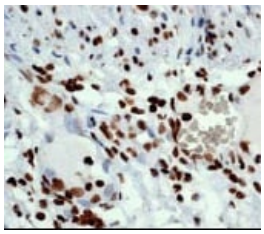
Negative Control 2: Mouse primary antibody(**ab7291**) and anti-rabbit secondary antibody(**ab150077**)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52946**).



Western blot - Anti-Histone H3 (acetyl K14) antibody [EP964Y] - BSA and Azide free (ab203952)

This data was developed using **ab52946**, the same antibody clone in a different buffer formulation. Different batches of **ab52946** were tested on C6 (Rat glial tumor glial cell) treated with Trichostatin A lysate at 1.2 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 15 kDa.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (acetyl K14) antibody [EP964Y] - BSA and Azide free (ab203952)

ab52946 at 1/100 dilution staining Histone H3 (acetyl K14) in human uterus adenocarcinoma tissue by Immunohistochemistry, Paraffin embedded tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab52946**).

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Anti-Histone H3 (acetyl K14) antibody [EP964Y] - BSA and Azide free (ab203952)

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