abcam

Product datasheet

Anti-Histone H3 (phospho S10) antibody ab5176

56 Abreviews 241 References 6 Images

Overview

Product name	Anti-Histone H3 (phospho S10) antibody
Description	Rabbit polyclonal to Histone H3 (phospho S10)
Host species	Rabbit
Specificity	This antibody is specific for phosho S10 of histone H3. We believe that it does not recognise the non-modified histone - no blocking is seen with the non-phospho peptide (see Western blot image).
Tested applications	**Suitable for:** WB, IHC-P, ICC
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Hamster, Xenopus laevis, Drosophila melanogaster, Apteronotus leptorhynchus, Cyanidioschyzon merolae, Hydractinia echinata
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as ab11477)
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab5176 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
WB	★★★★☆ (19)	1/5000. Detects a band of approximately 17 kDa (predicted molecular weight: 17 kDa).Can be blocked with Human Histone H3 (phospho S10) peptide (ab11477).
IHC-P	★★★★★ (6)	Use a concentration of 5 µg/ml.
ICC	★★★★★ (1)	Use at an assay dependent concentration.

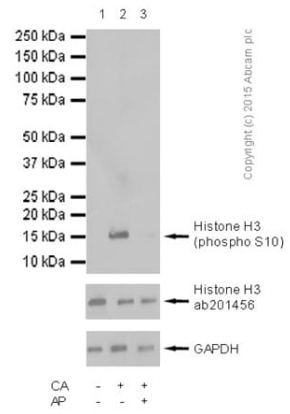
Target

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



Western blot - Anti-Histone H3 (phospho S10) antibody - ChIP Grade (ab5176)

All lanes : Anti-Histone H3 (phospho S10) antibody (ab5176) at 1/5000 dilution

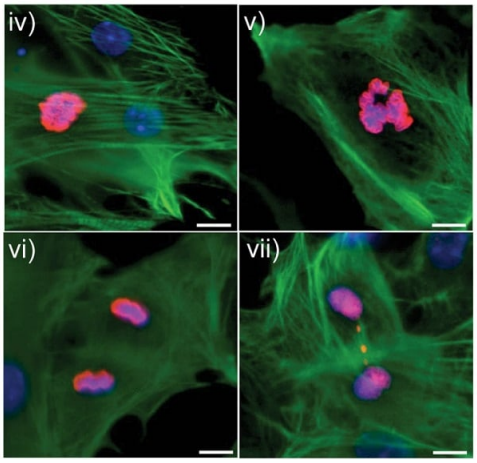
Lane 1 : Untreated HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa cell lysate treated with calyculin A
Lane 3 : HeLa cell lysate treated with calyculin A and alkaline phosphatase

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP goat anti-rabbit (H+L) at 1/20000 dilution

Predicted band size: 17 kDa

Exposure time: 1 second

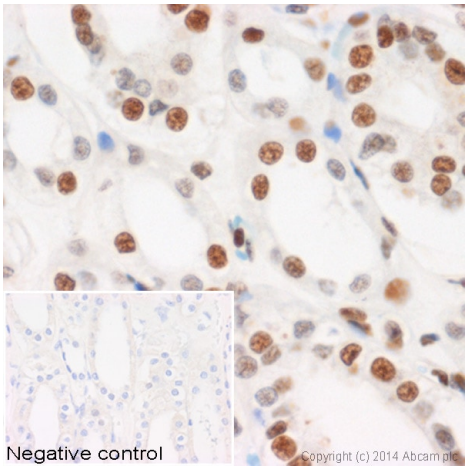


Immunocytochemistry - Anti-Histone H3 (phospho S10) antibody (ab5176)

Yamanaka S et al. Enhanced proliferation of monolayer cultures of embryonic stem (ES) cell-derived cardiomyocytes following acute loss of retinoblastoma. PLoS ONE 3:e3896 (2008).

Images of mouse cardiomyocyte in various stages of mitosis. Puromycin-resistant cells were stained with Hoechst 33342 (blue), and probed with antibodies specific to cTnT (Green) and Histone H3 (phospho S10) (Red) using ab5176 at 1/200 dilution in ICC/IF.

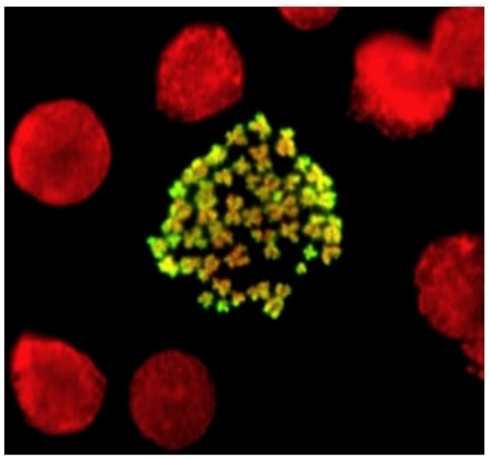
Mitotic activity was confirmed by staining with an antibody to phospho-H3 Histone, which is only phosphorylated at Ser10 during mitosis. Mitotic nuclei are pink (red+blue). Bar=10 µm.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (phospho S10) antibody - ChIP Grade (ab5176)

IHC image of ab5176 staining Histone H3 (phospho S10) in human kidney formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab5176, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. No primary antibody was used in the negative control (shown on the inset).

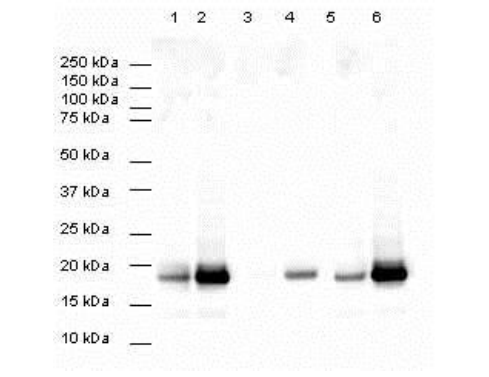
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry - Anti-Histone H3 (phospho S10) antibody (ab5176)

Image courtesy of Dr. Bryan Turner, United Kingdom

This image was kindly supplied by Prof Bryan Turner, University of Birmingham. Female Human Lymphoblastoid cells were incubated with ab5176.



Western blot - Anti-Histone H3 (phospho S10) antibody - ChIP Grade (ab5176)

All lanes : Anti-Histone H3 (phospho S10) antibody (ab5176) at 1 µg/ml

Lane 1 : untreated histones
Lane 2 : colcemid treated histones
Lane 3 : untreated histones with Human Histone H3 (phospho S10) peptide (ab11477) at 1 µg/ml
Lane 4 : colcemid treated histones with Human Histone H3 (phospho S10) peptide (ab11477) at 1 µg/ml
Lane 5 : untreated histones with Human Histone H3 (unmodified) peptide (ab2903) at 1 µg/ml
Lane 6 : colcemid treated histones with Human Histone H3 (unmodified) peptide (ab2903) at 1 µg/ml

Lysates/proteins at 0.5 µg per lane.

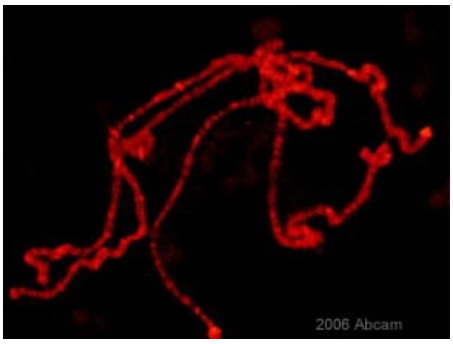
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 17 kDa

Exposure time: 10 seconds



Immunocytochemistry - Anti-Histone H3 (phospho S10) antibody (ab5176)

This image is courtesy of an Abreview submitted by Miss Anita Ciurciu

ab5176 at a 1/100 dilution staining Drosophila melanogaster (wild type) polytene chromosomes by ICC/IF. The cells were formaldehyde fixed and blocked with 1% BSA prior to incubation with the antibody for 12 hours. Bound antibody was detected using a Cy3 conjugated goat anti-rabbit antibody.

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