

Product datasheet

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

★★★★★ 50 Abreviews 114 References 9 Images

Overview

Product name	Anti-Histone H3 (phospho S10) antibody - ChIP Grade
Description	Rabbit polyclonal to Histone H3 (phospho S10) - ChIP Grade
Host species	Rabbit
Specificity	This antibody is specific for phospho 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: ICC/IF, WB, IHC-P, IHC - Wholemount, IHC-Fr, Flow Cyt, ChIP, IHC (PFA fixed), IHC-FoFr
Species reactivity	Reacts with: Mouse, Rat, Hamster, Cow, Human, <i>Xenopus laevis</i> , <i>Drosophila melanogaster</i> , Indian muntjac, Zebrafish, African green monkey, <i>Apteronotus leptorhynchus</i> , <i>Cyanidioschyzon merolae</i> , <i>Hydractinia echinata</i> Predicted to work with: <i>Saccharomyces cerevisiae</i> , <i>Caenorhabditis elegans</i> , <i>Schizosaccharomyces pombe</i> , <i>Neurospora crassa</i> 
Immunogen	Synthetic peptide corresponding to Human Histone H3 aa 1-100 (phospho S10) conjugated to Keyhole Limpet Haemocyanin (KLH). Database link: P68431 (Peptide available as ab11477)
Positive control	Colcemid-treated HeLa histone Preparation; HeLa whole cell extract IHC-P: Human normal kidney FFPE tissue sections.

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

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
ICC/IF	★★★★★	Use at an assay dependent concentration.
WB	★★★★☆	1/500 - 1/1000. 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	★★★★★	Use a concentration of 5 µg/ml.
IHC - Wholemout	★★★★★	1/250. PubMed: 20144789
IHC-Fr	★★★★★	1/200. (see Abreview)
Flow Cyt	★★★★★	1/1000. ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP	★★★★☆	Use at an assay dependent concentration. PubMed: 20089855
IHC (PFA fixed)		Use at an assay dependent concentration. PubMed: 25359725
IHC-FoFr	★★★★☆	Use at an assay dependent concentration. PubMed: 19622634

Target

Function	Variant histone H3 which replaces conventional H3 in a wide range of nucleosomes in active genes. Constitutes the predominant form of histone H3 in non-dividing cells and is incorporated into chromatin independently of DNA synthesis. Deposited at sites of nucleosomal displacement throughout transcribed genes, suggesting that it represents an epigenetic imprint of transcriptionally active chromatin. 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 throughout the cell cycle independently of DNA synthesis.
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 PAD14 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.

Specifically enriched in modifications associated with active chromatin such as methylation at Lys-5 (H3K4me), Lys-37 and Lys-80. 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), which are linked to gene repression, are underrepresented. 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.

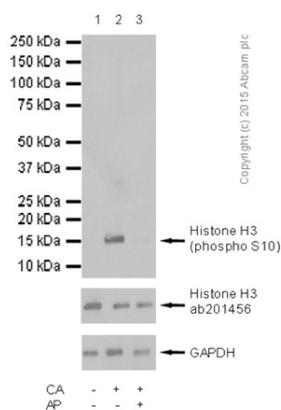
Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin. Phosphorylation on Ser-32 (H3S31ph) is specific to regions bordering centromeres in metaphase chromosomes.

Ubiquitinated. Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination.

Cellular localization

Nucleus. Chromosome.

Images



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

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

Lane 1 : Untreated HeLa cell lysate

Lane 2 : HeLa cell lysate treated with calyculin A

Lane 3 : HeLa cell lysate treated with calyculin A and alkaline phosphatase

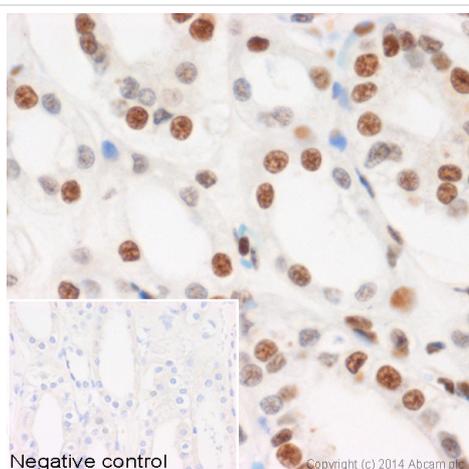
Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 17 kDa

Exposure time: 1 second

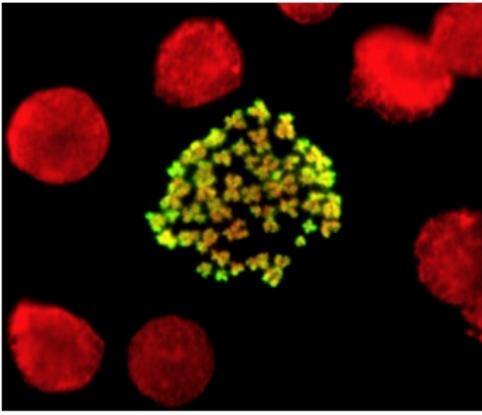


Negative control

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).

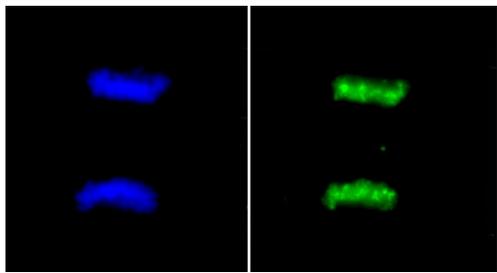
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



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

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10) antibody - ChIP Grade (ab5176)

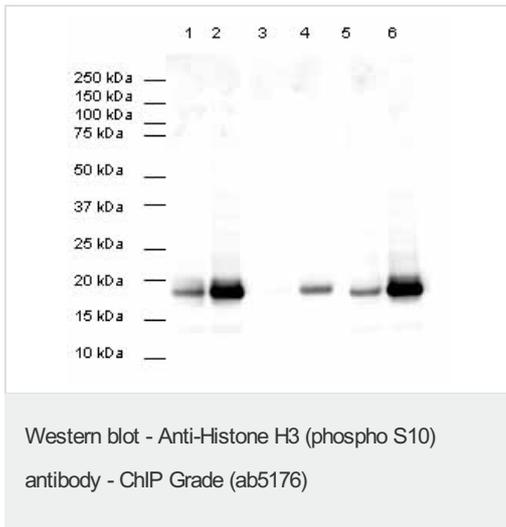
Image courtesy of Dr. Bryan Turner, United Kingdom



SKN cells stained with ab5176 (green) at a dilution of 1/100. The cells were fixed in paraformaldehyde for 10 minutes prior to incubation with ab5176. The DNA is stained with DAPI (blue). 100x magnification.

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10) antibody - ChIP Grade (ab5176)

This image is courtesy of Darin McDonald, Hendzel Laboratory



All lanes : Anti-Histone H3 (phospho S10) antibody - ChIP Grade (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

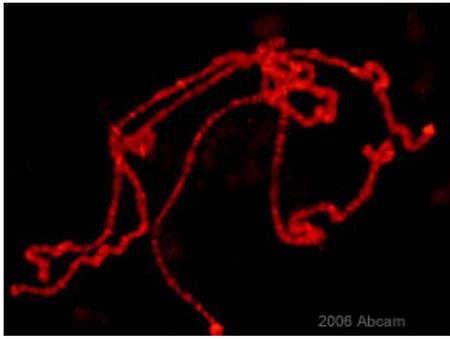
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (ab6721) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 17 kDa

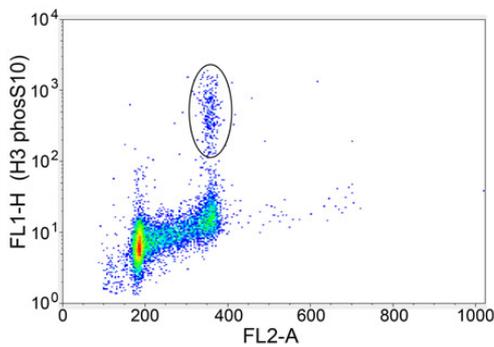
Exposure time: 10 seconds



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.

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S10) antibody - ChIP Grade (ab5176)

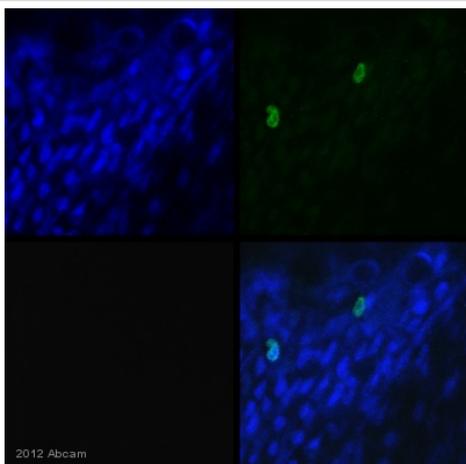
This image is courtesy of an Abreview submitted by Miss Anita Ciurciu



ab5176 (1/1000) staining a population of human HeLa cells positive for Histone H3 (phospho S10). Cells were trypsinized, pelleted and fixed in ice cold Ethanol. Debris was eliminated and FL2-A/FL2-W was used to eliminate clumping cells. For further experimental details please refer to abreview folder.

Flow Cytometry - Anti-Histone H3 (phospho S10) antibody - ChIP Grade (ab5176)

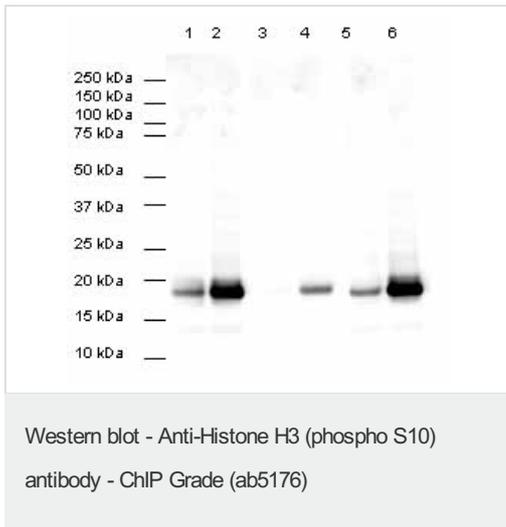
This image is courtesy of an Abreview submitted by Dr Kirk McManus



IHC-Wholemound image of Histone H3 (phospho S10) ab5176 on zebrafish jaw. 5 day old zebrafish were permeabilized with proteinase K (10ug/ml, 50min, 37degrees), prior to staining. Staining visible in mitotic cells. Image shows 2 cells labelled with H3 phospho S10 antibody (green), nuclei are labelled with DAPI (blue).

IHC - Wholemound - Anti-Histone H3 (phospho S10) antibody - ChIP Grade (ab5176)

This image was taken from an abreview by Chrissy Hammond.



Lysates: Lane 1,3,5: 0.5µg/lane untreated histone Lane 2,4,6: 0.5µg/lane colcemid treated histone - treated Primary antibody: Lane 1-2 : ab5176 (1?g/ml) Lane 3-4 :ab5176 (1?g/ml) + phospho peptide (ab5176) 1?g/ml Lane 5-6 :ab5176 (1?g/ml) + non-phospho peptide (ab2903 batch 3) 1?g/ml

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