abcam

Product datasheet

Anti-Histone H3 (tri methyl K9, phospho S10) antibody ab5819

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Overview

Product name	Anti-Histone H3 (tri methyl K9, phospho S10) antibody
Description	Rabbit polyclonal to Histone H3 (tri methyl K9, phospho S10)
Host species	Rabbit
Specificity	From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch, please contact our Scientific Support who will be happy to help.
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Cow, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Calf Thymus Histone, HeLa Nuclear, NIH3T3 Nuclear. ICC/IF: SKN cells, HeLa cells. IHC-P: Human breast carcinoma.
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 ab5819 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
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB		1/500 - 1/1000. Detects a band of approximately 22 kDa (predicted molecular weight: 17 kDa).
ICC/IF	★★★★☆ (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.

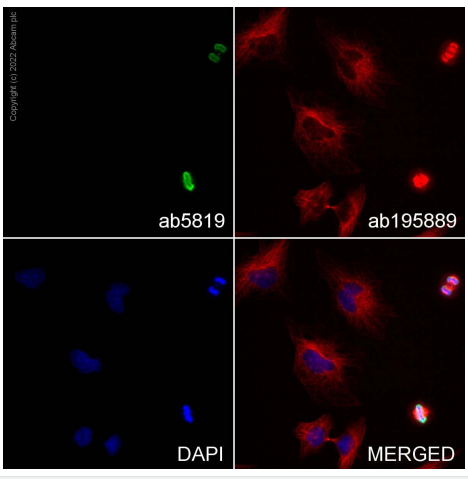
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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.

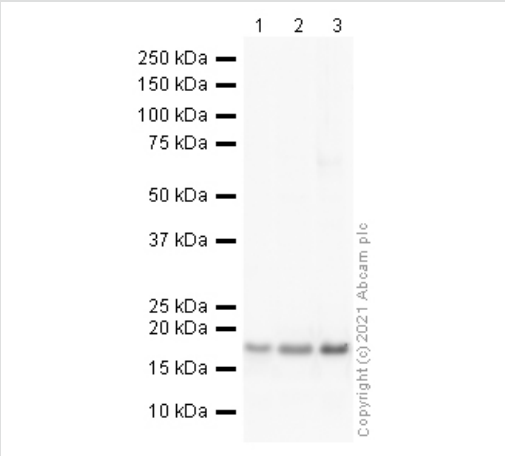
Images



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K9, phospho S10) antibody (ab5819)

ab5819 staining Histone H3 (tri methyl K9, phospho S10) in mitotic HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab5819 at 0.5µg/ml and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at 2µg/ml (shown in red). The secondary antibody (shown in green) was **ab150081**, Alexa Fluor® 488 Goat anti-Rabbit IgG (H+L) used at a 1/1000 dilution for 1h at room temperature. Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Western blot - Anti-Histone H3 (tri methyl K9, phospho S10) antibody (ab5819)

All lanes : Anti-Histone H3 (tri methyl K9, phospho S10) antibody (ab5819) at 1 µg/ml

Lane 1 : Calf Thymus Histone at 0.5 µg
Lane 2 : HeLa Nuclear – Triton Prep at 10 µg
Lane 3 : NIH3T3 Nuclear – Triton Prep at 10 µg

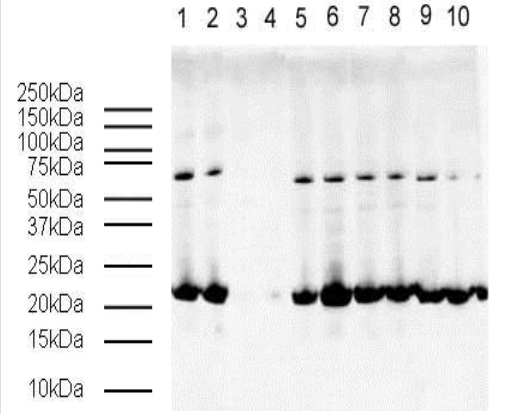
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

Predicted band size: 17 kDa
Observed band size: 17 kDa

Exposure time: 2 minutes

Blocking buffer: 2% BSA

Gel type: MES



Western blot - Anti-Histone H3 (tri methyl K9, phospho S10) antibody (ab5819)

All lanes : Anti-Histone H3 (tri methyl K9, phospho S10) antibody (ab5819) at 1/500 dilution

Lane 1 : HeLa histones
Lane 2 : Colcemid-treated HeLa histones
Lane 3 : HeLa histones with Human Histone H3 (tri methyl K9, phospho S10) peptide (**ab15644**) at 1 µg
Lane 4 : Colcemid-treated HeLa histones with Human Histone H3 (tri methyl K9, phospho S10) peptide (**ab15644**) at 1 µg
Lane 5 : HeLa histones with Human Histone H3 (unmodified) peptide (**ab7228**) at 1 µg
Lane 6 : Colcemid-treated HeLa histones with Human Histone H3 (unmodified) peptide (**ab7228**) at 1 µg
Lane 7 : HeLa histones with Human Histone H3 (phospho S10) peptide (**ab11477**) at 1 µg
Lane 8 : Colcemid-treated HeLa histones with Human Histone H3 (phospho S10) peptide (**ab11477**) at 1 µg
Lane 9 : HeLa histones with Human Histone H3 (tri methyl K9) peptide (**ab1773**) at 1 µg
Lane 10 : Colcemid-treated HeLa histones with Human Histone H3 (tri methyl K9) peptide (**ab1773**) at 1 µg

Lysates/proteins at 0.5 µg per lane.

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

Performed under reducing conditions.

Predicted band size: 17 kDa
Observed band size: 22 kDa
Additional bands at: 60 kDa. We are unsure as to the identity of these extra bands.

Rabbit polyclonal to Histone H3 (phospho S10, tri methyl K9) - ab5819, on histone preparations from HeLa cells. Primary antibody used at 1/500.

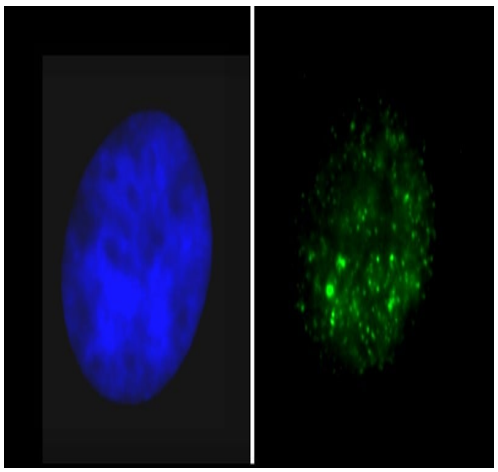
Lanes 1, 3, 5, 7, 9 - control HeLa histones (0.5ug/lane)
Lanes 2, 4, 6, 8, 10 - Colcemid-treated HeLa histones (0.5ug/lane)

All blocking peptides are 1ug/lane

Lanes 1 and 2: ab5819
Lanes 3 and 4: ab5819 + Histone H3 (Tri Me K9, phospho S10) peptide - **ab15644**
Lanes 5 and 6: ab5819 + Histone H3 peptide (unmodified) - **ab7228**
Lanes 7 and 8: ab5819 + Histone H3 (phospho S10) peptide - **ab11477**
Lanes 9 and 10: ab5819 + Histone H3 (tri methyl K9) peptide - **ab1773**

Secondary antibody - Goat polyclonal to rabbit IgG (HRP) - **ab6721** 1/5000.

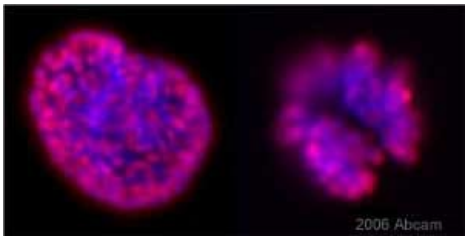
ab5819 is specific for Histone H3 phosphorylated at residue S10 and tri methylated at residue K9. The activity of the antibody is blocked by the addition of the immunizing peptide, **ab15644** (lanes 3 and 4).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K9, phospho S10) antibody - ChIP Grade (ab5819)

This image is courtesy of Darin McDonald, Hendzel Laboratory

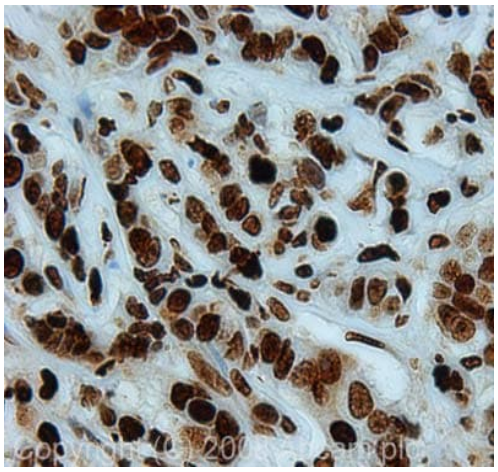
SKN cells stained with ab5819 (green) at a dilution of 1/2000. The cells were fixed in paraformaldehyde for 10 minutes prior to incubation with ab5819. The DNA is stained with DAPI (blue). 100x magnification.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K9, phospho S10) antibody - ChIP Grade (ab5819)

ab5819 at a 1/500 dilution staining mitotic human HeLa cells by immunocytochemistry. The antibody was incubated with formaldehyde fixed cells for 1 1/2 hours and bound antibody was then detected using a Texas red conjugated goat anti-rabbit IgG.

This image is courtesy of an Anonymous Abreview submitted on **6 April 2006.**



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K9, phospho S10) antibody - ChIP Grade (ab5819)

IHC image of Histone H3 (tri methyl K9, phospho S10) staining in human breast carcinoma FFPE section, performed on a Bond™ system using the standard protocol F. 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 ab5819, 5µg/ml, for 8 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.

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