abcam

Product datasheet

Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade ab14955

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Overview

Product name	Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade
Description	Mouse monoclonal [mAbcam 14955] to Histone H3 (tri methyl K9, phospho S10) - ChIP Grade
Host species	Mouse
Specificity	ab14955 recognises phospho S10 on Histone H3, even when K9 is tri methylated. It recognises a phospho S28 peptide by ELISA, but is not blocked by phospho S28 in WB.
Tested applications	**Suitable for:** IHC-P, ELISA, ICC/IF, ChIP, IP, IHC-Fr, Flow Cyt, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human, Xenopus laevis, Arabidopsis thaliana, Drosophila melanogaster, Indian muntjac, African green monkey, Oncopeltus **Predicted to work with:** Chicken, Saccharomyces cerevisiae, Caenorhabditis elegans, Schizosaccharomyces pombe, Zebrafish, Mammals, Tobacco, Chlamydomonas reinhardtii, Aspergillus nidulans, Neurospora crassa
Immunogen	Synthetic peptide within Human Histone H3 aa 1-100 (tri methyl K9, phospho S10) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary. (Peptide available as ab11477, ab15644)
Positive control	WB: Control HeLa Histone Prep. Colcemid treated HeLa Histone prep. HeLa cell lysate treated with calyculin A. IHC-P: Human kidney tissue.
General notes	This antibody clone is manufactured by Abcam. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact orders@abcam.com or you can find further information here. Hybridomas were prepared and the resulting clones were positively screened by ELISA against the immunising tri methyl K9 and phospho S10 dimodified peptide. Clones were also positively screened against both tri methyl K9 and phospho S10 peptides. Clones were negatively screened against the unmodified version of the peptides. This clone binds to the tri methyl K9 and phospho S10 dimodified peptide and to the phospho S10 peptide, but not to the tri methyl K9 peptide or to equivalent unmodified Histone H3 peptide.

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: 7.50 Preservative: 0.02% Sodium azide Constituent: PBS
Purity	IgG fraction
Clonality	Monoclonal
Clone number	mAbcam 14955
Isotype	IgG1

Applications

Our Abpromise guarantee covers the use of **ab14955** 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 at an assay dependent concentration.
ELISA		Use at an assay dependent concentration.
ICC/IF	★★★★☆	Use at an assay dependent concentration.
ChIP	★★★☆☆	Use at an assay dependent concentration. PubMed: 20864037
IP	★☆☆☆☆	Use at 80 µg/mg of lysate.
IHC-Fr	★★★★★	Use at an assay dependent concentration. PubMed: 21734301
Flow Cyt	★★★★☆	1/1000. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
WB	★★★★☆	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).

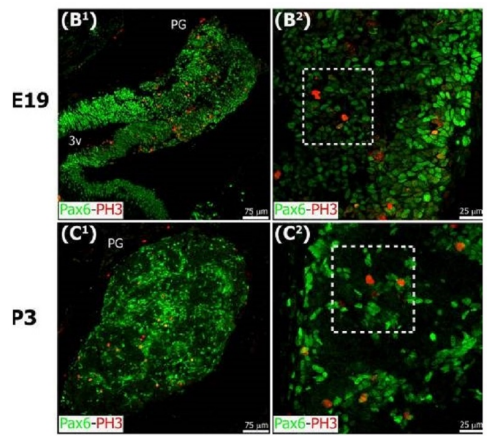
Target

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

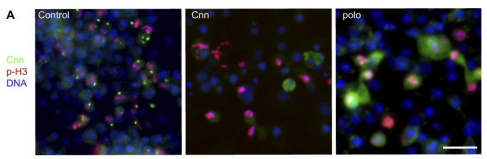


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

Ibañez Rodriguez et al PLoS One. 2016 Nov 18;11(11):e0167063. doi: 10.1371/journal.pone.0167063. eCollection 2016. Fig 4. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Confocal microscopy of sagittal sections through the rat pineal gland (PG) immunolabeled for Pax6 (green) and the mitotic M-phase marker phospho-histone H3 (PH3, ab14955, red). All PH3+ mitotic cells expressed Pax6; Pax6 levels, however, varied among dividing pinealocyte precursor cells. The number of mitotic precursor cells increased progressively during embryogenesis, and dropped quickly after birth. White arrowheads: Pax6^{high} mitoses. White arrows: Pax6^{low} mitoses.

E, embryonic day. PC, posterior commissure. P, postnatal day. SCO, subcommissural organ. 3v, third ventricle.

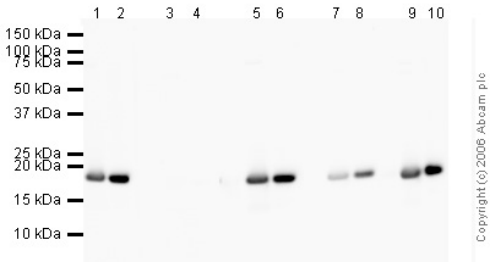


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

Dobbelaere et al PLoS Biol. 2008 Sep 16;6(9):e224. doi: 10.1371/journal.pbio.0060224. Fig 7. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Polo and Cnn Are Interdependent for Their Localisation and Function at Centrosomes

(A) Pictures from the primary screen of S2R+ cells treated with dsRNA against GFP (control), Cnn, and polo. The localisation of Cnn (green), Phospho-histone H3 (ab14955, 1/2000, red), and DNA (blue) is shown.



Western blot - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

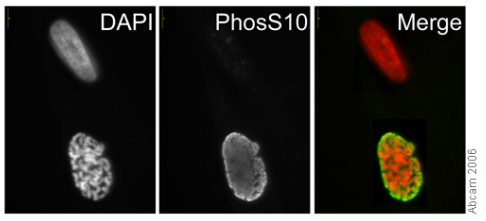
All lanes : Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955) at 1 µg/ml

Lane 1 : Control HeLa Histone Prep 0.5ug
Lane 2 : Colcemid treated HeLa Histone prep 0.5ug
Lane 3 : Control HeLa Histone Prep 0.5ug with Human Histone H3 (tri methyl K9, phospho S10) peptide (ab15644) at 1 µg/ml
Lane 4 : Colcemid treated HeLa Histone prep 0.5ug with Human Histone H3 (tri methyl K9, phospho S10) peptide (ab15644) at 1 µg/ml
Lane 5 : Control HeLa Histone Prep 0.5ug with Human Histone H3 (unmodified) peptide (ab7228) at 1 µg/ml
Lane 6 : Colcemid treated HeLa Histone prep 0.5ug with Human Histone H3 (unmodified) peptide (ab7228) at 1 µg/ml
Lane 7 : Control HeLa Histone Prep 0.5ug with Human Histone H3 (phospho S10) peptide (ab11477) at 1 µg/ml
Lane 8 : Colcemid treated HeLa Histone prep 0.5ug with Human Histone H3 (phospho S10) peptide (ab11477) at 1 µg/ml
Lane 9 : Control HeLa Histone Prep 0.5ug with Human Histone H3 (phospho S28) peptide (ab14793) at 1 µg/ml
Lane 10 : Colcemid treated HeLa Histone prep 0.5ug with Human Histone H3 (phospho S28) peptide (ab14793) at 1 µg/ml

Secondary
All lanes : Rabbit polyclonal to Mouse IgG H&L (HRP) at 1/5000 dilution

Performed under reducing conditions.

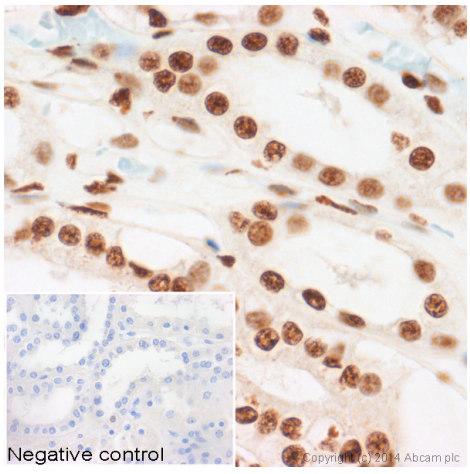
Predicted band size: 15 kDa
Observed band size: 17 kDa
why is the actual band size different from the predicted?



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

This image was submitted as part of a review by Kirk McManus

Indian muntjac and HeLa (Human epithelial cell line from cervix adenocarcinoma) cells were fixed in paraformaldehyde and labeled with ab14955 (1/2000 dilution) for 30 minutes. The image contains an interphase and a prophase Indian muntjac cells immunofluorescently labeled with anti-PhosS10 (green) and counterstained with DAPI (red).

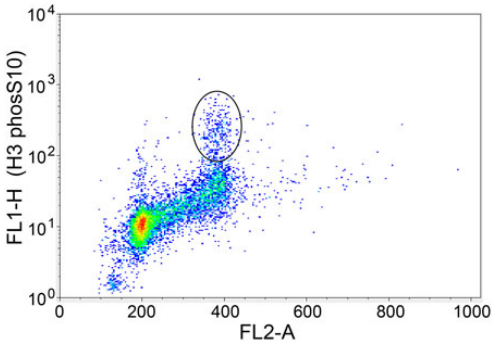


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

IHC image of ab14955 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 (pH 6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab14955, 1µg/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 hematoxylin and mounted with DPX. No primary antibody was used in the negative control (shown on the inset).

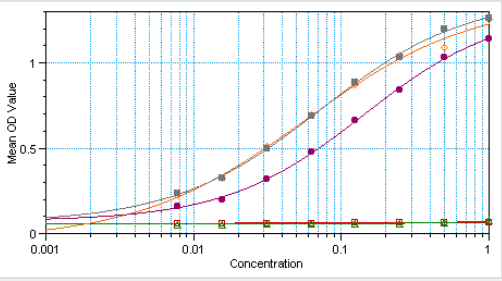
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Flow Cytometry - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

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

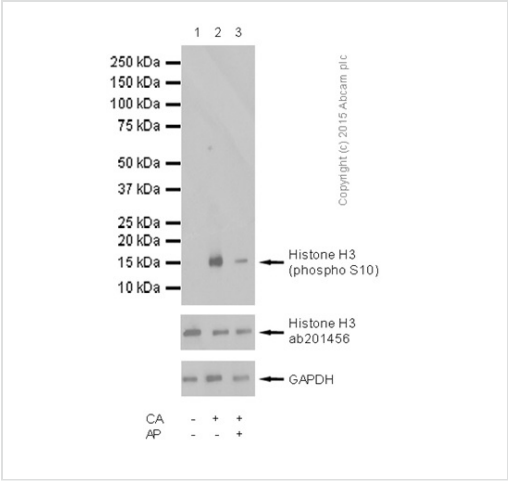
ab14955 (1/1000) stainning a population of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells positive for Histone H3 (phospho S10). Cells were trypsizined, pelleted and fixed in ice cold ethanol. Cellular debris was eliminated and the FL2-A/FL2-W was used to eliminate clumping cells. For further experimental details please refer to abreview.



ELISA - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

By ELISA, ab14955 detects:
the singly modified phospho S10 peptide and the dual modified phospho S10 and tri methyl K9 peptide (the orange and grey lines respectively),
less strongly detects the phospho S28 peptide (purple line),
does not detect the equivalent non-modified Histone H3 peptide for S10 or the singly modified tri methyl K9 peptide (the 2 lines at the bottom of the figure).

The antibody recognises phospho S28 by ELISA (although a phospho S28 peptide does not block the antibody in Western blotting).



Western blot - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

All lanes : Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955) at 1/2000 dilution

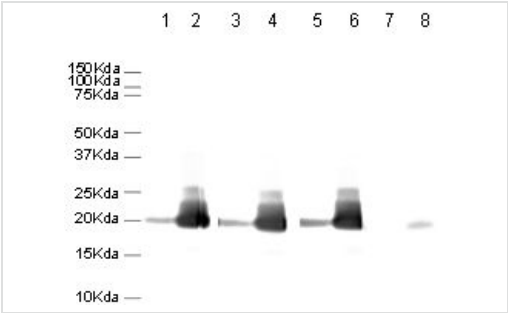
Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : HeLa cell lysate treated with calyculin A
Lane 3 : HeLa cell lysate treated with calyculin A and alkaline phosphatase

Lysates/proteins at 10 µg per lane.

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

Predicted band size: 15 kDa

Exposure time: 1 second



Western blot - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

All lanes : Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955) at 0.5 µg/ml

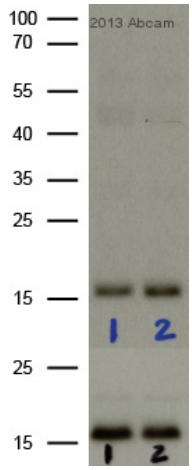
Lane 1 : Control HeLa Histone prep
Lane 2 : Colecemid treated HeLa Histone prep
Lane 3 : Control HeLa Histone prep with Human Histone H3 (unmodified) peptide (ab7228) at 1 µg/ml
Lane 4 : Colecemid treated HeLa Histone prep with Human Histone H3 (unmodified) peptide (ab7228) at 1 µg/ml
Lane 5 : Control HeLa Histone prep with Human Histone H3 (phospho S28) peptide (ab14793) at 1 µg/ml
Lane 6 : Colecemid treated HeLa Histone prep with Human Histone H3 (phospho S28) peptide (ab14793) at 1 µg/ml
Lane 7 : Control HeLa Histone prep with Human Histone H3 (phospho S10) peptide (ab11477) at 1 µg/ml
Lane 8 : Colecemid treated HeLa Histone prep with Human Histone H3 (phospho S10) peptide (ab11477) at 1 µg/ml

Lysates/proteins at 5 µg per lane.

Secondary
All lanes : Rabbit Anti-Mouse IgG H&L (HRP) (ab6728) at 1/5000 dilution

Predicted band size: 15 kDa

Observed band size: 20 kDa why is the actual band size different from the predicted?



Western blot - Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955)

Image courtesy of Richelle Sopko, Harvard University, U.S.A

All lanes : Anti-Histone H3 (tri methyl K9, phospho S10) antibody [mAbcam 14955] - ChIP Grade (ab14955) at 1/1000 dilution

Lane 1 : Wild type 0-4 hour old fruit fly embryo lysate

Lane 2 : 0-4 hour old fruit fly embryo lysate expressing wee RNAi

Secondary

All lanes : Anti-mouse IgG, peroxidase-linked at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Exposure time: 5 seconds

Blocking: 10% BSA

wee shRNA embryos (lane 2) should display elevated phospho H3Ser10 levels relative to wild type

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