abcam

Product datasheet

Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade ab10799

*** * * 17 Abreviews 128 References 7 Images

Overview

Product name Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade

Description Mouse monoclonal [mAbcam 10799] to Histone H3 - ChIP Grade

Host species Mouse

Tested applications Suitable for: ChIP, WB, IHC-P, IP

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Rat, Cow, Human, Drosophila melanogaster, Recombinant fragment

Predicted to work with: Monkey, a wide range of other species, Mammals

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as <u>ab12149</u>)

Positive control WB: HeLa, Calf Thymus Histone and NIH/3T3, whole cell lysates. PC12 nuclear lysate. IP: HeLa

whole cell extract; IHC-P: FFPE human breast adenocarcinoma.

General notes

This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or

conjugation for your experiments, please contact **orders@abcam.com**.

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 Constituents: PBS, 6.97% L-Arginine

1

Purity IgG fraction Clonality Monoclonal

Clone number mAbcam 10799

Myeloma Sp2/0-Ag14

Isotype lgG3 Light chain type kappa

Applications

Our Abpromise guarantee covers the use of ab10799 in the following tested applications. The Abpromise guarantee

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ChIP	★★★★ ★ <u>(2)</u>	Use 2-4 µg for 25 µg of chromatin.
WB	★★★★★ (12)	Use a concentration of 1 µg/ml. Detects a band of approximately 18 kDa (predicted molecular weight: 15 kDa). Can be blocked with Human Histone H3 peptide (ab12149) .
IHC-P	****(1)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IP		Use a concentration of 1 µg/ml.

Application notes Is unsuitable for ICC/IF.

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

Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs modifications 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 PAD4 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.

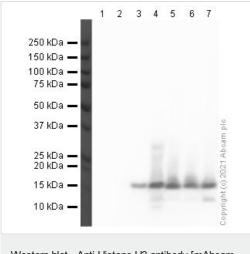
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and 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-iun. 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 antibody [mAbcam 10799] - ChIP Grade (ab10799)

All lanes : Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade (ab10799) at 5 µg/ml

Lane 1: Histone H2A Recombinant Protein at 0.1 µg/ml

Lane 2: Histone H2B Recombinant Protein at 0.1 µg/ml

Lane 3: Histone H3.1 Recombinant Protein at 0.1 µg/ml

Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5

µg/ml

Lane 5: HeLa nuclear lysate (triton enriched) at 10 µg/ml

Lane 6: NIH 3T3 nuclear lysate (triton enriched) at 10 µg/ml

Lane 7: PC12 nuclear lysate (triton enriched) at 10 µg/ml

Secondary

All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

Performed under reducing conditions.

Predicted band size: 15 kDa

Exposure time: 20 seconds

ChIP: ab10799

ChIP: ab10799

ab10799

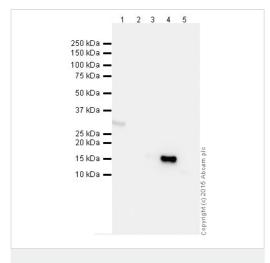
ab10799

active inactive heterochromatin

Every new batch of this antibody is tested at Abcam in ChIP.

ChIP - Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade (ab10799)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 2µg of ab10799 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.



Western blot - Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade (ab10799)

All lanes : Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade (ab10799) at 5 μ g/ml

Lane 1 : Histone H1 Recombinant Protein
Lane 2 : Histone H2A Recombinant Protein
Lane 3 : Histone H2B Recombinant Protein
Lane 4 : Histone H3.1 Recombinant Protein
Lane 5 : Histone H4 Recombinant Protein

Lysates/proteins at 0.1 µg per lane.

Secondary

All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/5000 dilution

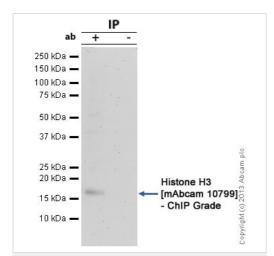
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Exposure time: 4 minutes

We recommend using 3% milk as the blocking agent in Western Blot.



Immunoprecipitation - Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade (ab10799)

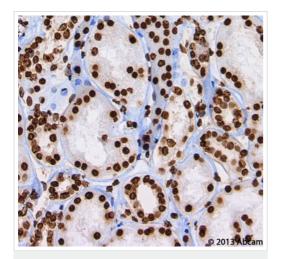
Histone H3 [mAbcam 10799] - ChIP Grade was immunoprecipitated using 0.5mg HeLa whole cell extract, 5µg of Mouse monoclonal to and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, HeLa whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of $40\mu l$ SDS loading buffer and incubated for 10min at $70^{\circ}C$; $10\mu l$ of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab 10799.

Secondary: Goat polyclonal to mouse IgG light chain specific (HRP) at 1/20,000 dilution.

Band: 15kDa; [mAbcam 10799] Histone H3 - ChlP Grade.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade (ab10799)

ab10799 staining human kidney sections by IHC-P using EXPOSE IHC detection kit (ab80436). Formalin fixed paraffin embedded tissue sections were pre-treated using heat mediated antigen retrieval (using a pressure cooker) with sodium citrate buffer (pH6) for 30 mins. The section was incubated with ab10799, $5\mu g/ml$, for 1 hour at room temperature. DAB was used as the chromogen and the section was counterstained with haematoxylin and mounted with DPX.



Western blot - Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade (ab10799)

All lanes : Anti-Histone H3 antibody [mAbcam 10799] - ChIP Grade (ab10799) at 1 µg/ml

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: Calf Thymus Histone preparation nuclear lysate

Lane 3: NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

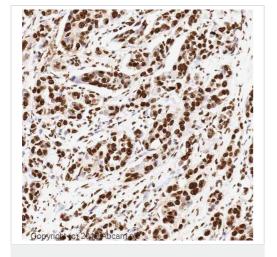
Secondary

All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 15 kDa **Observed band size:** 17 kDa

Exposure time: 90 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 antibody
[mAbcam 10799] - ChIP Grade (ab10799)

IHC image of Histone H3 staining in human breast carcinoma FFPE section, performed on a BondTM 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 ab10799, 1µg/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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