


Product datasheet

Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade ab31830

★★★★★ 4 Abreviews 44 References 8 Images

Overview

Product name	Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade
Description	Mouse monoclonal [mAbcam 31830] to Histone H4 - ChIP Grade
Host species	Mouse
Tested applications	Suitable for: Flow Cyt, IHC-P, IP, WB, ChIP, ICC/IF
Species reactivity	Reacts with: Cow, Human, Recombinant fragment Predicted to work with: Mouse, Rat 
Immunogen	Synthetic peptide corresponding to Human Histone H4 aa 50 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. Database link: P62805 (Peptide available as ab13843)
Positive control	This antibody gave a positive signal in the following lysates: Calf Thymus Histone Preparation Nuclear Lysate HeLa Histone Preparation Nuclear Lysate Histone H4 Recombinant Protein IHC-P: FFPE human breast fibroadenoma. ICC/IF: HeLa cell line
General notes	<p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>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.</p> <p>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</p>

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
Constituents: PBS, 6.97% L-Arginine

Purity	IgG fraction
Clonality	Monoclonal
Clone number	mAbcam 31830
Myeloma	Sp2/0-Ag14
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our [Abpromise guarantee](#) covers the use of ab31830 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
Flow Cyt		Use 1 µg for 10 ⁶ cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★★ (1)	Use a concentration of 0.05 - 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
WB	★★★★★ (2)	Use a concentration of 1 µg/ml. Detects a band of approximately 13 kDa (predicted molecular weight: 14 kDa). Can be blocked with Human Histone H4 peptide (ab13843) .
ChIP		Use 5 µg for 25 µg of chromatin.
ICC/IF		Use a concentration of 5 µg/ml.

Target

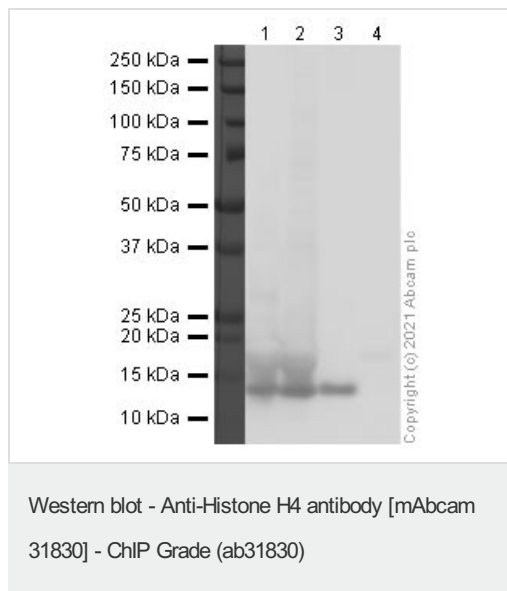
Function	Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA 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 H4 family.
Post-translational modifications	Acetylation at Lys-6 (H4K5ac), Lys-9 (H4K8ac), Lys-13 (H4K12ac) and Lys-17 (H4K16ac) occurs in coding regions of the genome but not in heterochromatin. Citullination at Arg-4 (H4R3ci) by PAD4 impairs methylation. Monomethylation and asymmetric dimethylation at Arg-4 (H4R3me1 and H4R3me2a, respectively) by PRMT1 favors acetylation at Lys-9 (H4K8ac) and Lys-13 (H4K12ac).

Demethylation is performed by JMJD6. Symmetric dimethylation on Arg-4 (H4R3me2s) by the PRDM1/PRMT5 complex may play a crucial role in the germ-cell lineage. Monomethylated, dimethylated or trimethylated at Lys-21 (H4K20me1, H4K20me2, H4K20me3). Monomethylation is performed by SET8. Trimethylation is performed by SUV420H1 and SUV420H2 and induces gene silencing. 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. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage. The exact role of H4K91ub1 in DNA damage response is still unclear but it may function as a licensing signal for additional histone H4 post-translational modifications such as H4 Lys-21 methylation (H4K20me). Sumoylated, which is associated with transcriptional repression.

Cellular localization

Nucleus. Chromosome.

Images



All lanes : Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg/ml

Lane 2 : HeLa Nuclear Lysate (Triton enriched) at 10 µg/ml

Lane 3 : Histone H4 Recombinant Protein at 0.1 µg/ml

Lane 4 : Histone H3.1 Recombinant Protein at 0.1 µg/ml

Secondary

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

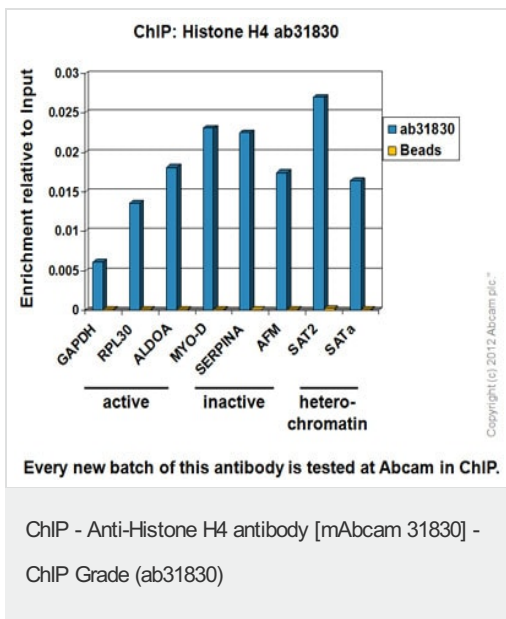
Developed using the ECL technique.

Performed under reducing conditions.

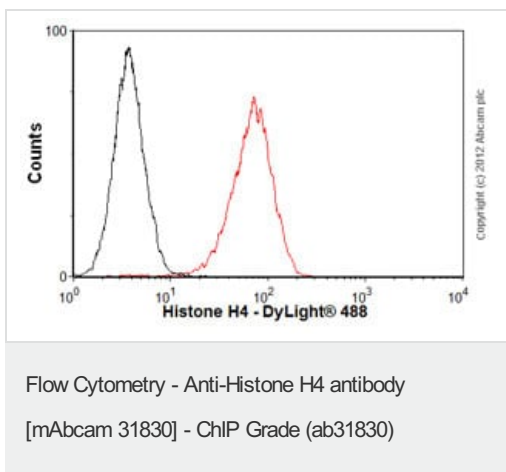
Predicted band size: 14 kDa

Additional bands at: 13 kDa. We are unsure as to the identity of these extra bands.

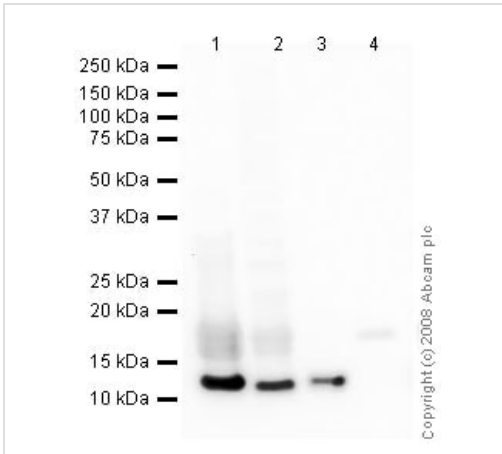
Exposure time: 10 seconds



Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab31830 (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.



Overlay histogram showing HeLa cells stained with ab31830 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab31830, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (ab91353, 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed.



Western blot - Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830)

All lanes : Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg

Lane 2 : HeLa Histone Preparation Nuclear Lysate at 2.5 µg

Lane 3 : Histone H4 Recombinant Protein at 0.1 µg

Lane 4 : Histone H3.1 Recombinant Protein at 0.1 µg

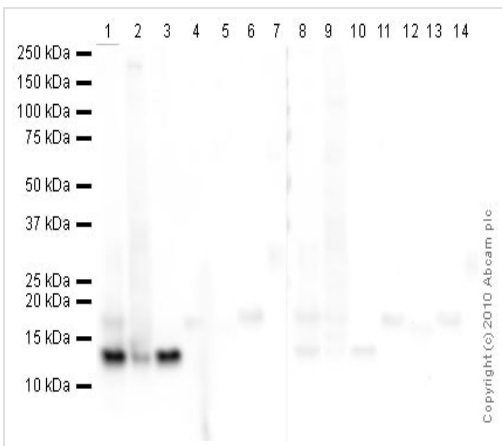
Secondary

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

Performed under reducing conditions.

Predicted band size: 14 kDa

Observed band size: 13 kDa



Western blot - Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830)

All lanes : Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830) at 5 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg

Lane 2 : HeLa Histone Preparation Nuclear Lysate at 2.5 µg

Lane 3 : Histone H4 Recombinant Protein at 0.1 µg

Lane 4 : Histone H3.1 Recombinant Protein at 0.1 µg

Lane 5 : Histone H2A Recombinant Protein at 0.1 µg

Lane 6 : Histone H2B Recombinant Protein at 0.1 µg

Lane 7 : Histone H1 Recombinant Protein at 0.1 µg

Lane 8 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg with Human Histone H4 peptide ([ab13843](#)) at 5 µg/ml

Lane 9 : HeLa Histone Preparation Nuclear Lysate at 2.5 µg with Human Histone H4 peptide ([ab13843](#)) at 5 µg/ml

Lane 10 : Histone H4 Recombinant Protein at 0.1 µg with Human Histone H4 peptide ([ab13843](#)) at 5 µg/ml

Lane 11 : Histone H3.1 Recombinant Protein at 0.1 µg with Human Histone H4 peptide ([ab13843](#)) at 5 µg/ml

Lane 12 : Histone H2A Recombinant Protein at 0.1 µg with Human Histone H4 peptide ([ab13843](#)) at 5 µg/ml

Lane 13 : Histone H2B Recombinant Protein at 0.1 µg with Human Histone H4 peptide ([ab13843](#)) at 5 µg/ml

Lane 14 : Histone H1 Recombinant Protein at 0.1 µg with Human Histone H4 peptide ([ab13843](#)) at 5 µg/ml

Secondary

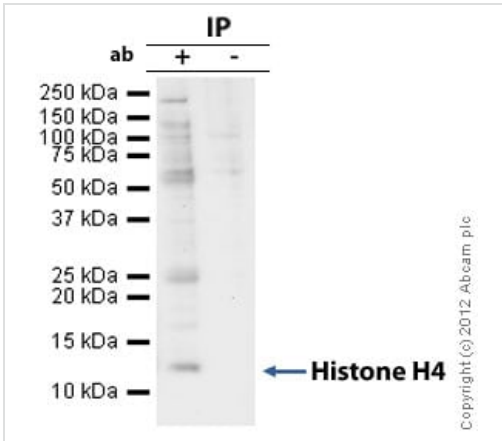
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 14 kDa

Observed band size: 13 kDa

Exposure time: 2 minutes



Immunoprecipitation - Anti-Histone H4 antibody
[mAbcam 31830] - ChIP Grade (ab31830)

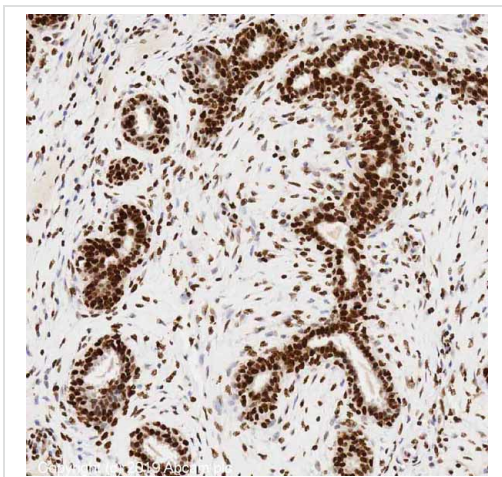
Histone H4 was immunoprecipitated using 0.5mg HeLa whole cell extract, 10µg of Mouse monoclonal to Histone H4 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

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 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab31830.

Secondary: Protein G-HRP at 1/500 dilution.

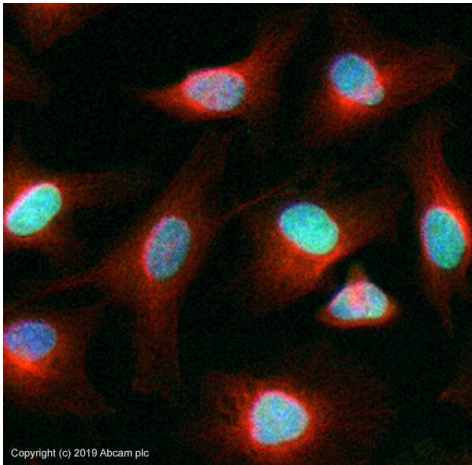
Band: 13kDa: Histone H4; non specific - 25 and 55kDa: We are unsure as to the identity of this extra band.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 antibody
[mAbcam 31830] - ChIP Grade (ab31830)

IHC image of Histone H4 staining in human breast

fibroadenoma formalin fixed paraffin embedded tissue section, performed on a Leica 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 [ab31380](#), 0.1µg/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 haematoxylin and mounted with DPX.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830)

ab31830 stained in HeLa cells. Cells were fixed with 4% paraformaldehyde (10min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% Triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab31830 at 5µg/ml and ab6046 (Rabbit polyclonal to beta Tubulin - Loading Control) used at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150117, Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed, (pseudo-colored green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) preadsorbed, (colored red), both used at a 1/1000 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1hour at room temperature.

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