## abcam

### Product datasheet

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

\*\*\* \* \* 6 Abreviews 51 References 7 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 (Intra), IHC-P, WB, ChIP, ICC/IF

Unsuitable for: IP

Species reactivity Reacts with: Cow, Human

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

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.

1

Storage buffer pH: 7.50

Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine

Purity lgG fraction
Clonality Monoclonal

**Clone number** mAbcam 31830 **Myeloma** Sp2/0-Aq14

**lsotype** lgG1 **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 (Intra)		Use 1µg for 10 <sup>6</sup> cells.  ab170190 - Mouse monoclonal lgG1, is suitable for use as an isotype control with this antibody.
IHC-P	**** <u>(1)</u>	Use a concentration of 0.05 - 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
WB	<b>★★★★★ (4)</b>	Use a concentration of 1 µg/ml. Detects a band of approximately 13 kDa (predicted molecular weight: 14 kDa). Can be blocked with <b>Human Histone H4 peptide (ab13843)</b> .
ChIP		Use 5 µg for 25 µg of chromatin.
ICC/IF		Use a concentration of 5 μg/ml.

**Application notes** Is unsuitable for IP.

### **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.

Citrullination at Arg-4 (H4R3ci) by PADI4 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.

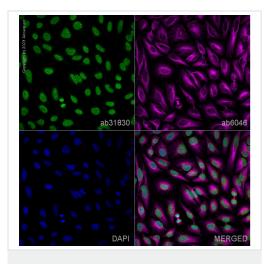
Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage. The exact role of H4K91ub1 in 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**

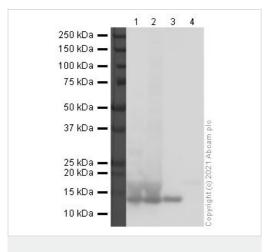


Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830)

ab31830 staining Histone H4 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-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 ab31830 at 1µg/ml and ab6046, Rabbit polyclonal to beta Tubulin - Loading Control. Cells were then incubated with ab150117, Goat polyclonal Secondary Antibody to Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (shown in green) and ab150080, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 594) at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

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 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/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

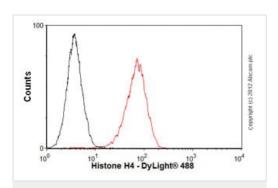
ChIP: Histone H4 ab31830

ChIP: Histone H4 ab31830

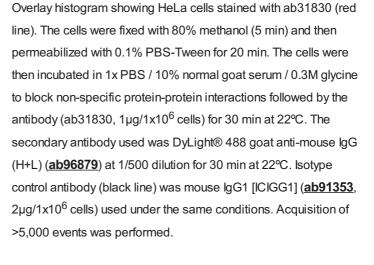
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ChIP - Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830)

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.



Flow Cytometry (Intracellular) - Anti-Histone H4 antibody [mAbcam 31830] - ChIP Grade (ab31830)





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

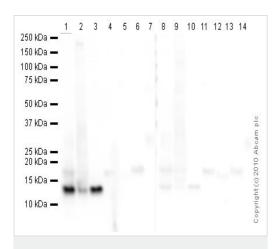
**Lane 3 :** Histone H4 Recombinant Protein at 0.1 μg **Lane 4 :** Histone H3.1 Recombinant Protein at 0.1 μg

#### 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  $\mu g$ 

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  $\mu$ g 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

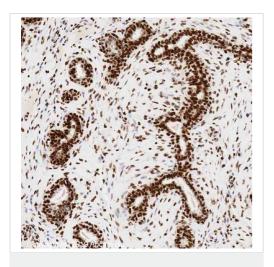
All lanes : Goat polyclonal to Mouse IgG - H&L - Pre-Adsorbed

(HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 14 kDa
Observed band size: 13 kDa

Exposure time: 2 minutes



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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<sup>TM</sup> 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.

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