# abcam

## Product datasheet

## Anti-Histone H4 (acetyl K8) antibody - ChIP Grade ab15823

★★★★★ 5 Abreviews 70 References 画像数 5

製品の概要

製品名 Anti-Histone H4 (acetyl K8) antibody - ChIP Grade

製品の詳細 Rabbit polyclonal to Histone H4 (acetyl K8) - ChIP Grade

由来種 Rabbit

アプリケーション 適用あり: IHC-P, ChIP, WB, ICC/IF

種交差性 交差種: Cow, Human

交差が予測される動物種: Mouse, Chicken, Saccharomyces cerevisiae

免疫原 Synthetic peptide corresponding to Human Histone H4 aa 1-100 (internal sequence) (acetyl K8)

conjugated to Keyhole Limpet Haemocyanin (KLH).

Database link: P62805

(Peptide available as ab15824)

ポジティブ・コントロール ChIP: Chromatin was prepared from U2OS cells. IHC-P: Human breast adenocarcinoma tissue.

WB: Calf Thymus Histone Preparation Nuclear Lysate. ICC/IF: HeLa cells.

特記事項

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

製品の特性

製品の状態 Liquid

保存方法 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.

**バッファー** 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.

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精製度 Immunogen affinity purified

**ポリ/モノ** ポリクローナル

アイソタイプ IgG

#### アプリケーション

**The Abpromise guarantee** <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab15823の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ChIP	<b>★★★★★ (2)</b>	Use 2µl for 10 <sup>6</sup> cells.
WB	**** <u>(1)</u>	Use a concentration of 1 µg/ml. Detects a band of approximately 12 kDa (predicted molecular weight: 11.2 kDa).
ICC/IF	<b>★★★☆☆(2)</b>	Use a concentration of 0.1 - 1 µg/ml.

#### ターゲット情報

機能 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.

配列類似性 Belongs to the histone H4 family.

翻訳後修飾 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.

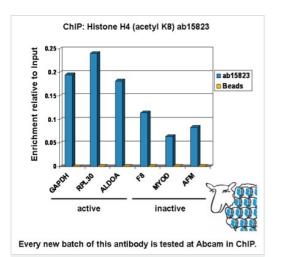
Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage.

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).

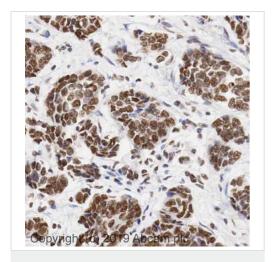
Sumoylated, which is associated with transcriptional repression.

細胞内局在 Nucleus, Chromosome.



ChIP - Anti-Histone H4 (acetyl K8) antibody - ChIP Grade (ab15823)

Chromatin was prepared from U2OS cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25  $\mu g$  of chromatin, 2  $\mu g$  of ab15823 (blue), and 20  $\mu l$  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). Primers and probes are located in the first kb of the transcribed region.

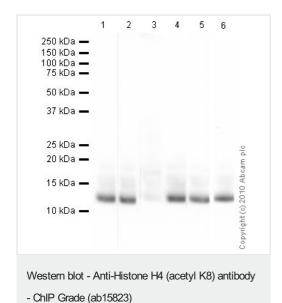


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H4 (acetyl K8) antibody - ChIP Grade (ab15823)

IHC image of histone H4 (acetyl K8) antibody staining in a section of formalin-fixed paraffin-embedded human breast adenocarcinoma\* performed on a Leica BOND<sup>TM</sup> system using the standard protocol. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab15823, 0.5ug/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.

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.

\*Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



All lanes : Anti-Histone H4 (acetyl K8) antibody - ChIP Grade (ab15823) at 1  $\mu$ g/ml

Lane 1: Calf Thymus Histone Preparation Nuclear Lysate (ab121)

Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate (ab121)

with Human Histone H4 peptide ( $\underline{ab15825}$ ) at 0.5  $\mu g/ml$ 

**Lane 3 :** Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H4 (acetyl K8) peptide (<u>ab15824</u>) at 0.5 µg/ml

Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate (ab121)

with Histone H4 peptide - acetyl K5 at 0.5  $\mu g/ml$ 

**Lane 5 :** Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H4 (acetyl K12) peptide (<u>ab15662</u>) at 0.5 µg/ml

**Lane 6 :** Calf Thymus Histone Preparation Nuclear Lysate (<u>ab121</u>) with Human Histone H2A (acetyl K9) peptide (<u>ab54016</u>) at 0.5  $\mu$ g/ml

Lysates/proteins at 0.5 µg/ml per lane.

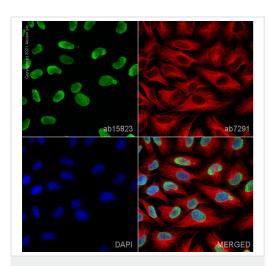
#### **Secondary**

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

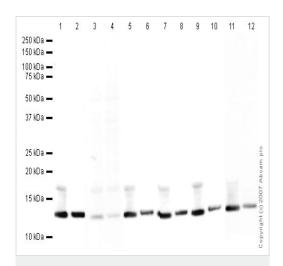
Performed under reducing conditions.

**Predicted band size:** 11.2 kDa **Observed band size:** 13 kDa

Exposure time: 3 minutes



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (acetyl K8) antibody - ChIP Grade (ab15823)



Western blot - Anti-Histone H4 (acetyl K8) antibody - ChIP Grade (ab15823)

ab15823 staining Histone H4 (Acetyl K8) 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 ab15823 at 0.1 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor<sup>®</sup> 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor<sup>®</sup> 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). 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.

**All lanes :** Anti-Histone H4 (acetyl K8) antibody - ChIP Grade (ab15823) at 1 µg/ml

Lane 1: Sodium butyrate treated hela histone

Lane 2: Untreated hela histone

**Lane 3 :** Sodium butyrate treated hela histone with Human Histone H4 (acetyl K8) peptide (**ab15824**) at 1 μg/ml

**Lane 4 :** Untreated hela histone with Human Histone H4 (acetyl K8) peptide (ab15824) at 1  $\mu$ g/ml

**Lane 5 :** Sodium butyrate treated hela histone with Human Histone H4 peptide (ab15825) at 1  $\mu$ g/ml

**Lane 6 :** Untreated hela histone with Human Histone H4 peptide ( $\underline{ab15825}$ ) at 1  $\mu g/ml$ 

Lane 7: Sodium butyrate treated hela histone with Histone H4 peptide - acetyl K5 (ab15596) at 1 µg/ml

**Lane 8 :** Untreated hela histone with Histone H4 peptide - acetyl K5 (ab15596) at 1  $\mu$ g/ml

**Lane 9 :** Sodium butyrate treated hela histone with Human Histone H4 (acetyl K12) peptide (<u>ab15662</u>) at 1  $\mu$ g/ml

**Lane 10 :** Untreated hela histone with Human Histone H4 (acetyl K12) peptide (ab15662) at 1  $\mu$ g/ml

Lane 11: Sodium butyrate treated hela histone with Histone H2A

peptide - acetyl K9 at 1 µg/ml

Lane 12: Untreated hela histone with Histone H2A peptide - acetyl

K9 at 1 µg/ml

Lysates/proteins at 2.5 µg per lane.

#### Secondary

All lanes: IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at

1/10000 dilution

Performed under reducing conditions.

**Predicted band size:** 11.2 kDa **Observed band size:** 12 kDa

Additional bands at: 22 kDa (possible cross reactivity)

The blot shows that a H4 peptide containing an acetylated K8 residue was able to block recognition of histones by ab15823. Peptides containing other acetylated K residues of H4 and H2A did not block binding of histones by ab15823. This suggests ab15823 specifically recognises Histone H4 acetyl K8.

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