# abcam

# Product datasheet

# Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade ab40888

重组Rok

RabMAb

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概述

产品名称 Anti-Histone H3 (acetyl K18)抗体[EP959Y] - ChIP Grade

**宿主** Rabbit

特异性 This antibody only detects Histone H3 when acetylated on Lysine 18.

经测试应用 适用于: ICC/IF, ChIP, ChIP-sequencing, WB, IHC-P

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide within Human Histone H3 aa 1-100 (acetyl K18). The exact sequence is

proprietary.

**阳性**对照 WB: C6, NIH/3T3 and HeLa cells treated with 500ng/ml Trichostatin A for 4 hours. IHC-P: Human

breast carcinoma and endometrium cancer tissue. Mouse liver and rat stomach tissue. ICC/IF: HeLa cells. ChIP: Chromatin prepared from HeLa cells. ChIP-seq: Chromatin prepared from

HeLa cells.

常规说明 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

性能

形式 Liquid

**存放说明** Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

**存储溶液** pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

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纯**度** Protein A purified

**同种型** IgG

应用

# The Abpromise guarantee Abpromise™承诺保证使用ab40888于以下的经测试应用

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ICC/IF	***** <u>(1)</u>	1/1000. For unpurified use at 1/100-1/250
ChIP		Use 5 µg for 25 µg of chromatin.
ChIP-sequencing		Use 4µg for 10 <sup>7</sup> cells.
WB		1/20000. Detects a band of approximately 17 kDa (predicted molecular weight: 17 kDa). <b>For unpurified use at 1/500</b>
IHC-P		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

### 靶标

功能 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.

序列相似性 Belongs to the histone H3 family.

发展阶段 Expressed during S phase, then expression strongly decreases as cell division slows down

during the process of differentiation.

翻译后修饰 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 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-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.

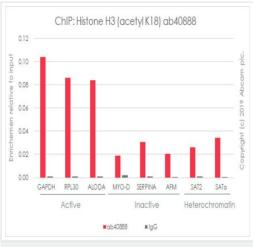
细胞定位 Nucleus. Chromosome.

图片

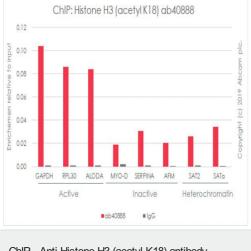
# 6,500 kb 6,525 kb 6,550 kb 6,575 kb Chromosome 12 (active region) Input ab40888 ╫╫╫╫ Refseq Genes IFF01 NCAPD2 NOP2 SCARNA10 Copyright (c) 2021 Abcam plc. ChIP-sequencing - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

Chromatin was prepared from HeLa cells. Cells were fixed with 1% formaldehyde for 10 minutes. ChIP was performed with  $10^7$  HeLa cells and 4  $\mu$ g of ab40888 [EP959Y]. ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

Additional screenshots of mapped reads can be downloaded **here**.



ChIP - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)



2 199 kBa = 50 kDa -37 kDa -25 kDa -20 kDa -- Histone H3 (acetyl K18) - ChIP Grade 15 kDa -10 kDa --Histone H3 (acetyl K18) - ChIP Grade (ab177870) - GAPDH (ab181602) Trichostatin A: -

Western blot - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

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 25  $\mu g$  of chromatin, 5  $\mu g$  of ab40888 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). 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.

All lanes: Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888) at 1/20000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates

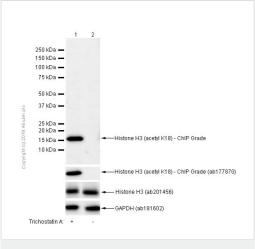
Lane 2: HeLa (Human cervix adenocarcinoma epithelial cell) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysates

Lysates/proteins at 15 µg per lane.

# Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 17 kDa Observed band size: 17 kDa

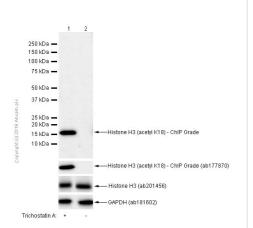


Western blot - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

250 kDa — 150 kDa — 100 kDa 🕳 75 kDa -

50 kDa -37 kDa 🕳

25 kDa — 20 kDa —



-Histone H3 (acetyl K18) - ChIP Grade (ab177870)

Western blot - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

- Histone H3 (acetyl K18) - ChIP Grade

-Histone H3 (ab201456) — GAPDH (ab181602)

All lanes: Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888) at 1/50000 dilution (Purified)

All lanes: Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP

Lane 2: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000

Lane 1: NIH/3T3 (Mouse embryonic fibroblast) treated with

500ng/ml Trichostatin A for 4 hours whole cell lysates

Grade (ab40888) at 1/50000 dilution (Purified)

Lysates/proteins at 15 µg per lane.

Predicted band size: 17 kDa Observed band size: 17 kDa

**Secondary** 

dilution

Lane 1: C6 (Rat glial tumor glial cell) treated with 500ng/ml Trichostatin A for 4 hours whole cell lysates

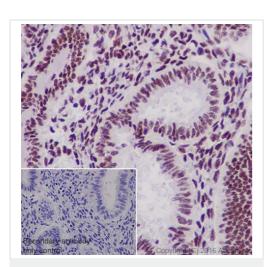
Lane 2: C6 (Rat glial tumor glial cell) whole cell lysates

Lysates/proteins at 15 µg per lane.

## Secondary

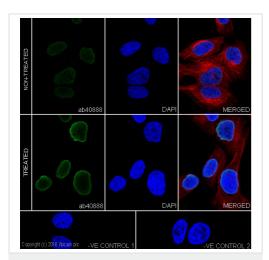
All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 17 kDa Observed band size: 17 kDa



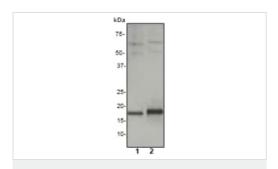
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human endometrium cancer tissue sections labeling Histone H3 with purified ab40888 at 1/4000 dilution (0.23 µg/ml). Perform heat mediated antigen retrieval using <a href="mailto:ab93684">ab93684</a> (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) treated with 500ng/ml Trichostatin A for 4 hours cells labeling Histone H3 with purified ab40888 at 1/1000 dilution (0.9 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) at 1/200 (2.5 µg/ml) dilution. Goat anti rabbit lgG (Alexa Fluor<sup>®</sup> 488, ab150077) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Western blot - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

**All lanes :** Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888) at 1/50000 dilution

Lane 1: C6 cell lysate, untreated.

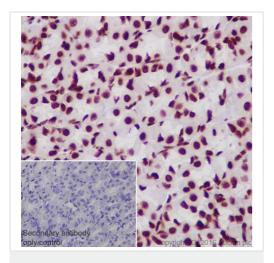
Lane 2: C6 cell lysate, treated with TSA.

Lysates/proteins at 10 µg per lane.

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

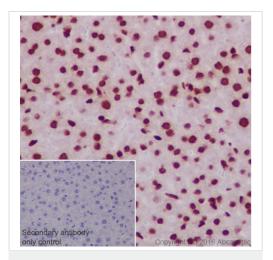
Additional bands at: 50 kDa (possible non-specific binding), 60

kDa (possible non-specific binding)



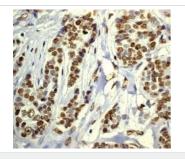
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat stomach tissue sections labeling Histone H3 with purified ab40888 at 1/4000 dilution (0.23  $\mu$ g/ml). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



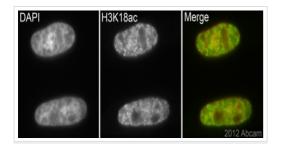
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver tissue sections labeling Histone H3 with purified ab40888 at 1/4000 dilution (0.23  $\mu$ g/ml). Perform heat mediated antigen retrieval using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

ab40888 diluted 1:100, staining acetylated histone H3 on human breast carcinoma sections.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K18) antibody [EP959Y] - ChIP Grade (ab40888)

Image courtesy of an Abreview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MICB, Canada

ab40888 (1/500) staining Histone H3 (acetyl K18) in asynchronous HeLa cells (green). Cells were fixed in paraformaldehyde, permeabilised with 0.5% Triton X-100/PBS and counterstained with DAPI in order to highlight the nucleus (red). For further experimental details please refer to Abreview.



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