# abcam

#### Product datasheet

## Anti-Histone H3 (acetyl K27) antibody - ChIP Grade ab4729

★★★★★ 84 Abreviews 1912 References 9 图像

概述

免疫原

产品名称 Anti-Histone H3 (acetyl K27)抗体- ChIP Grade

描述 兔多克隆抗体to Histone H3 (acetyl K27) - ChIP Grade

**宿主** Rabbit

特异性 From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and

expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch,

please contact our Scientific Support who will be happy to help.

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

种属反应性 与反应: Mouse, Rat, Cow, Human, Recombinant fragment

预测可用于: Chicken, Xenopus laevis, Arabidopsis thaliana, Drosophila melanogaster, Monkey,

Zebrafish, Plasmodium falciparum, Rice, Cyanidioschyzon merolae

Synthetic peptide corresponding to Human Histone H3 aa 1-100 (acetyl K27) conjugated to

keyhole limpet haemocyanin. (Peptide available as <u>ab24404</u>)

**阳性**对照 WB: HeLa (human cervix adenocarcinoma epithelial cell) cell lysate - Sodium butyrate-treated,

HeLa (human cervix adenocarcinoma epithelial cell) nuclear lysate (triton enriched), NIH/3T3 (mouse embryonic fibroblast cell line) nuclear lysate (triton enriched) and PC-12 (rat adrenal gland

pheochromocytoma cell) nuclear lysate (triton enriched). ICC/IF: HeLa cells

常规说明 Learn about ChIP assay kits, other ChIP antibodies, protocols and more in the <u>ChIP assay</u>

guide.

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

性能

形式 Liquid

1

存放说明 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.

**存储溶液** 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.

纯**度** Immunogen affinity purified

#### 应用

### The Abpromise guarantee Abpromise™承诺保证使用ab4729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	<b>★★★★★</b> (23)	Use a concentration of 0.5 µg/ml. Can be used with paraformaldehyde- or methanol- fixed cells.
WB	**** (20)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).  We recommend <b>Goat Anti-Rabbit IgG H&amp;L (HRP) (ab97051) secondary antibody</b> .
IHC-P	**** (4)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ChIP	*** ( <u>27)</u>	Use 2 µg for 25 µg of chromatin.  We recommend GAPDH positive control ChIP primer pair <u>ab267832</u> as a positive control.
PepArr		Use a concentration of 0.2 - 0.02 μg/ml.

#### 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.

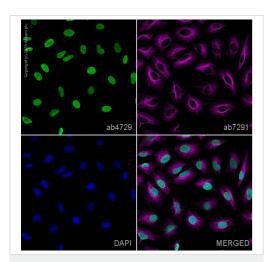
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and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

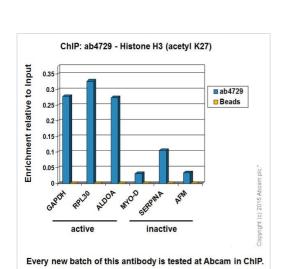
细胞定位

Nucleus. Chromosome.

图片



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729)



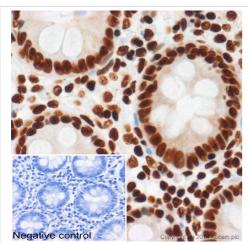
ChIP - Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729)

ab4729 staining Histone H3 (acetyl K27) 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 ab4729 at 1µg/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®488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG -H&L (Alexa Fluor® 594), pre-adsorbed 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.

Chromatin was prepared from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 µg of chromatin, 2 µg of ab4729 (blue), and 20 µl of Protein A/G sepharose beads.

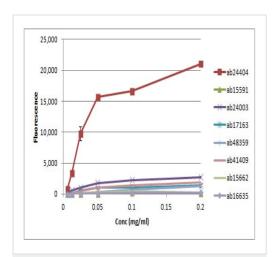
No antibody was added to the beads control (yellow).

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 paraffinantibody - ChIP Grade (ab4729)

embedded sections) - Anti-Histone H3 (acetyl K27)



Peptide Array - Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729)

IHC image of ab4729 staining Histone H3 (acetyl K27) in human colon formalin-fixed paraffin-embedded tissue sections\*, performed on a Leica Bond.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer pH 6 for 20 minutes. The section was then incubated with ab4729, 5 µg/ml, for 15 minutes 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.

No primary antibody was used in the negative control (shown on the inset).

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 batches of ab4729 are tested in Peptide Array against peptides to different Histone H3 modifications. Six dilutions of each peptide are printed on to the Peptide Array in triplicate and results are averaged before being plotted on to a graph. Results show strong binding to Histone H3 - acetyl K27 peptide (ab24404), indicating that this antibody specifically recognises the Histone H3 - acetyl K27 modification.

ab24404 - Histone H3 - acetyl K27

ab15591 - Histone H3 - acetyl K14

ab24003 - Histone H3 - acetyl K18

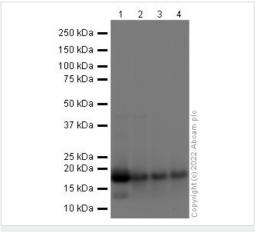
ab17163 - Histone H3 unmodified

ab48359 - Histone H3 - acetyl K23

ab41409 - Histone H3 - acetyl K36



ab16635 - Histone H3 acetyl K9



Western blot - Anti-Histone H3 (acetyl K27) antibody

- ChIP Grade (ab4729)

**All lanes :** Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729) at 1  $\mu$ g/ml

**Lane 1 :** HeLa (human cervix adenocarcinoma epithelial cell) cell lysate - Sodium butyrate-treated

**Lane 2 :** HeLa (human cervix adenocarcinoma epithelial cell) nuclear lysate (triton enriched)

**Lane 3**: NIH/3T3 (mouse embryonic fibroblast cell line) nuclear lysate (triton enriched)

**Lane 4 :** PC-12 (rat adrenal gland pheochromocytoma cell) nuclear lysate (triton enriched)

Lysates/proteins at 10 µg per lane.

#### **Secondary**

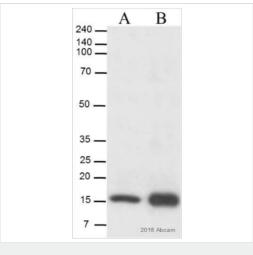
**All lanes :** Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

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

Exposure time: 30 seconds

Blocking buffer: 2% BSA block

Gel type: MES



Western blot - Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729)

**All lanes :** Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729) at 1/2500 dilution

Lane 1: Untreated Mouse MEF cell lysate

**Lane 2 :** 0.4  $\mu$ M Trichostatin A treatment for 18 hr Mouse MEF cell

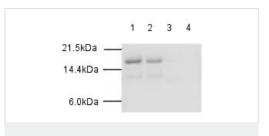
lysate

Lysates/proteins at 9 µg per lane.

#### Secondary

**All lanes :** Donkey Anti-Rabbit lgG H&L (HRP) (<u>ab6802</u>) at 1/20000 dilution

Predicted band size: 15 kDa



Western blot - Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729)

Lanes 1 & 3 : Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729) at 0.2  $\mu g/ml$ 

Lanes 2 & 4: Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729) at 0.1 µg/ml

Lanes 1-2: Calf thymus histone lysate

**Lanes 3-4 :** Calf thymus histone lysate with Human Histone H3 (acetyl K27) peptide (**ab24404**) at 2 μg

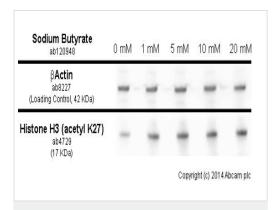
Lysates/proteins at 1 µg per lane.

#### **Secondary**

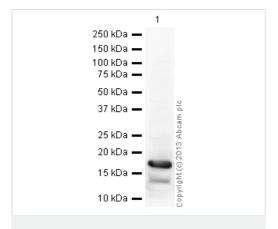
All lanes: Goat anti-rabbit (HRP) at 1/2000 dilution

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

ab4729 specifically recognises acetyl K27 histone H3 in catlf thymus histone lysate, which is specifically blocked using the immunizing peptide <u>ab24404</u>.



Western blot - Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729)



Western blot - Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729)

HeLa (Human epithelial cell line from cervix adenocarcinoma) cells were incubated at  $37^{\circ}$ C for 6 hours with vehicle control (0  $\mu$ M) and different concentrations of sodium butyrate (<u>ab120948</u>). Increased expression of histone H3 (acetyl K27)(ab4729) in HeLa cells correlates with an increase in sodium butyrate concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 2.5 µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab4927 at 1 µg/ml and ab8227 at 1 µg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10,000 dilution and visualised using ECL development solution.

Anti-Histone H3 (acetyl K27) antibody - ChIP Grade (ab4729) at 1  $\mu$ g/ml + HeLa (Human epithelial cell line from cervix adenocarcinoma) histone preparation, nuclear Lysate - Butyrate treated at 2.5  $\mu$ g

#### **Secondary**

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Additional bands at: 17 kDa. We are unsure as to the identity of

these extra bands.

Exposure time: 10 seconds

#### Our Abpromise to you: Quality guaranteed and expert technical support

- · Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.cn/abpromise">https://www.abcam.cn/abpromise</a> or contact our technical team.

#### Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors