abcam

Product datasheet

Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade ab8580

★★★★★ 90 Abreviews 1982 References 7 图像

概述

产品名称	Anti-Histone H3 (tri methyl K4)抗体- ChIP Grade
描述	兔多克隆抗体to Histone H3 (tri methyl K4) - ChIP Grade
宿主	Rabbit
经测试应用	**适用于:** PepArr, ChIP, WB, IHC-P, ICC/IF
种属反应性	**与反应:** Cow, Human **预测可用于:** Mouse, Rat, Rabbit, Pig, Saccharomyces cerevisiae, Tetrahymena, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis elegans, Drosophila melanogaster, Indian muntjac, Oikopleura, Plants, Zebrafish, Mammals, Trypanosoma cruzi, Common marmoset, Rice, Xenopus tropicalis
免疫原	Synthetic peptide within Human Histone H3 aa 1-100 (tri methyl K4) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary. (Peptide available as **ab92374**)
阳性对照	ICC/IF: HeLa cells
常规说明	In immunofluorescence, a distinct property of tri methyl lysine 4 is its apparent 'ringing' of regions that appear as nucleoplasmic 'holes'. These represent the positions of splicing factor compartments, which often are easy to identify using only DNA stains in Indian muntjac fibroblasts. These splicing factor compartments are known to be preferentially associated with active genes and highly acetylated histone H3. This antibody, as expected, fails to stain heterochromatin (work by Kirk McManus, lab of Michael Hendzel). The immunofluorescence results suggest this antibody is **an exceptional euchromatin probe**. Learn about ChIP assay kits, other ChIP antibodies, protocols and more in the **ChIP assay guide**. Abcam recommended secondaries - Goat Anti-Rabbit HRP (**ab205718**) and Goat Anti-Rabbit Alexa Fluor® 488 (**ab150077**). See other **anti-rabbit secondary antibodies** that can be used with this antibody. 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
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
克隆	**多克隆**
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab8580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
PepArr		Use a concentration of 0.2 - 0.02 µg/ml. Slight cross reactivity is observed with the Histone H3 - di methyl K4 modification. Optimisation is recommended to avoid array signal saturation.
ChIP	★★★★★ (28)	Use 2 µg for 25 µg of chromatin. We recommend GAPDH positive control ChIP primer pair **ab267832** as positive control.
WB	★★★★★ (20)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
IHC-P	★★★★★ (8)	Use at an assay dependent concentration.
ICC/IF	★★★★★ (24)	Use a concentration of 1 µg/ml. 1/100 - 1/5000

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.

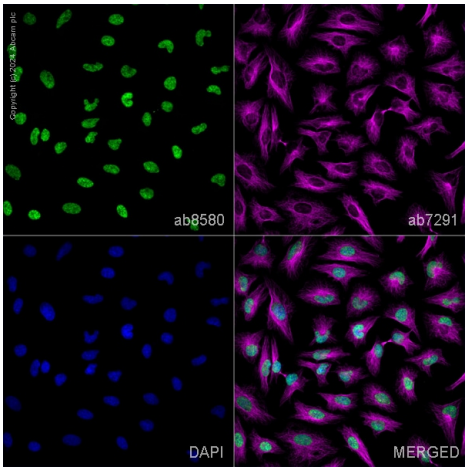
发展阶段 Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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 PADI4 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.

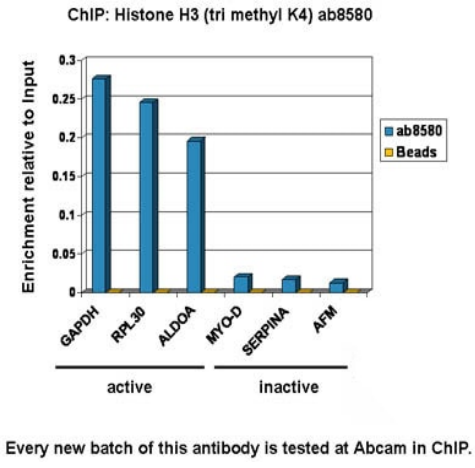
图片

图片



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580)

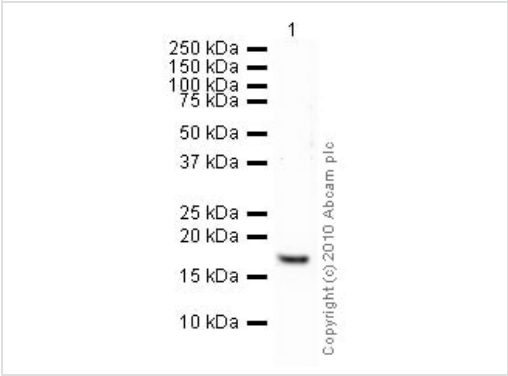
ab8580 staining Histone H3 (tri methyl K4) in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab8580 at 0.1µg/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.



ChIP - Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580)

Chromatin was prepared from U-2 OS (Human bone osteosarcoma epithelial cell line) 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 ab8580 (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.



Western blot - Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580)

Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580) at 1 µg/ml + Calf thymus histone preparation (nuclear lysate) at 0.5 µg

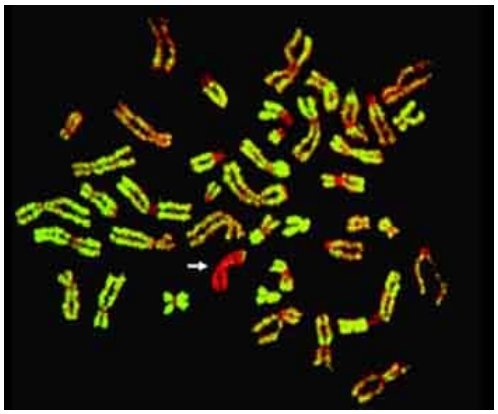
Secondary

Goat Anti-Rabbit IgG (H+L) HRP- conjugated antibody at 1/50000 dilution

Performed under reducing conditions.

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

Exposure time: 8 minutes

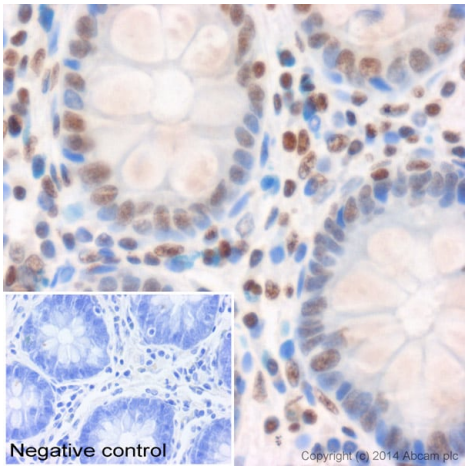


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580)

This image is courtesy of Ahmad Khalil and Daniel Driscoll, University of Florida College of Medicine.

Human female lymphoblast immunostained with ab8580 (1:100) (yellowish green) specific for histone H3 lysine 4 (H3-K4) trimethylation; the DNA is stained red with propidium iodide (PI).

Note the inactive X chromosome (arrow) and pericentromeric heterochromatin are largely devoid of this modification.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580)

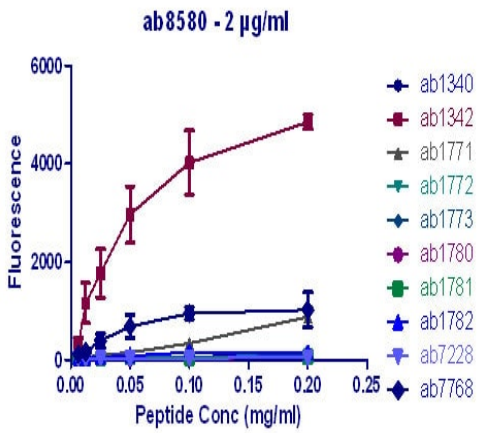
IHC image of ab8580 staining Histone H3 (tri methyl K4) in human colon formalin-fixed paraffin-embedded tissue sections*, performed on a Leica Bond.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab8580, 1/500 dilution, 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 (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



Peptide Array - Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580)

All batches of ab8580 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 - tri methyl K4 peptide (**ab1342**), indicating that this antibody specifically recognises the Histone H3 - tri methyl K4 modification. Slight cross reactivity is observed with the Histone H3 - di methyl K4 modification. Optimization is recommended to avoid array signal saturation.

ab1340 - Histone H3 - mono methyl K4

ab1342 - Histone H3 - tri methyl K4

ab1771 - Histone H3 - mono methyl K9

ab1772 - Histone H3 - di methyl K9

ab1773 - Histone H3 - tri methyl K9

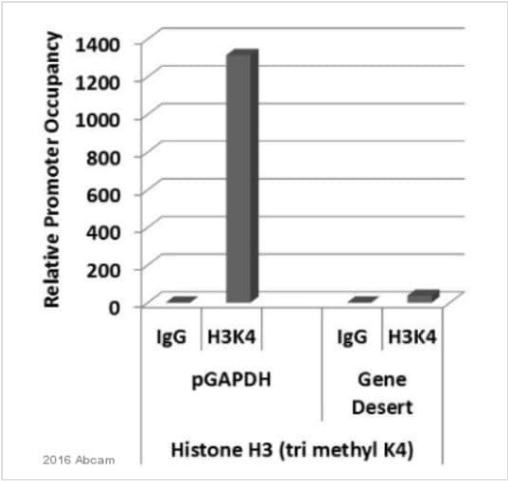
ab1780 - Histone H3 - mono methyl K27

ab1781 - Histone H3 - di methyl K27

ab1782 - Histone H3 - tri methyl K27

ab7228 - Histone H3 - unmodified

ab7768 - Histone H3 - di methyl K4



ChIP - Anti-Histone H3 (tri methyl K4) antibody - ChIP Grade (ab8580)

This image is courtesy of an anonymous Abreview

Chromatin was prepared from human cell lysate - nuclear B cells according to the Abcam X-ChIP protocol.

Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 0.5 µg of ab8580 per µg chromatin in ChIP Buffer fot 16 hours at 4°C. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region. Negative control: IgG and Gene Desert. Positive control: GAPDH Promoter.