abcam

Product datasheet

Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade ab176842

重组 RabMAb

★★★★★ 13 Abreviews 60 References 10 图像

概述

产品名称	Anti-Histone H3抗体[EPR16987] -核Marker and ChIP Grade
描述	兔单克隆抗体[EPR16987] to Histone H3 -核Marker and ChIP Grade
宿主	Rabbit
经测试应用	**适用于:** IHC-P, ICC/IF, PepArr, WB, ChIP, Flow Cyt (Intra)
种属反应性	**与反应:** Mouse, Rat, Human, Saccharomyces cerevisiae, Drosophila melanogaster, Schizosaccharomyces pombe **预测可用于:** Sheep, Dog, a wide range of other species, Common marmoset
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HeLa, NIH3T3, Saccharomyces cerevisiae and Schizosaccharomyces pombe whole cell lysates. Drosophila embryo nuclear extract lysate. IHC-P: Human colon, Mouse liver, and Rat pancreas tissues. ICC/IF: HeLa cells. ChIP: 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® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® 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 long term. Avoid freeze / thaw cycle.
存储溶液	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified

克隆	单克隆
克隆编号	EPR16987
同种型	IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab176842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
IHC-P	★★★★★ (6)	1/5000 - 1/10000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF	★★★★☆ (1)	1/2000 - 1/5000.
PepArr		Use at an assay dependent concentration.
WB	★★★★★ (5)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
ChIP		Use 2 µg for 25 µg of chromatin.
Flow Cyt (Intra)		Use at an assay dependent concentration.

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.

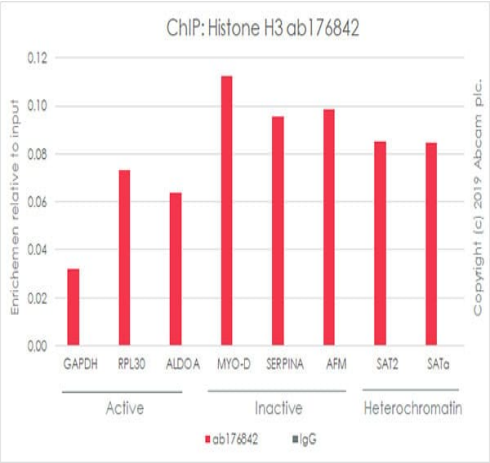
发展阶段 Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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.

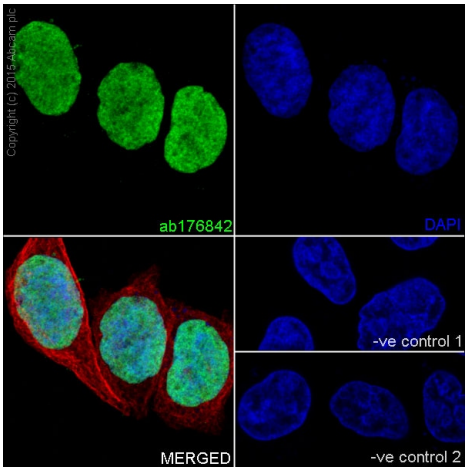
细胞定位 Nucleus. Chromosome.

图片



ChIP - Anti-Histone H3 antibody [EPR16987] - ChIP Grade (ab176842)

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, 2µg of ab176842 (red), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (grey). 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.



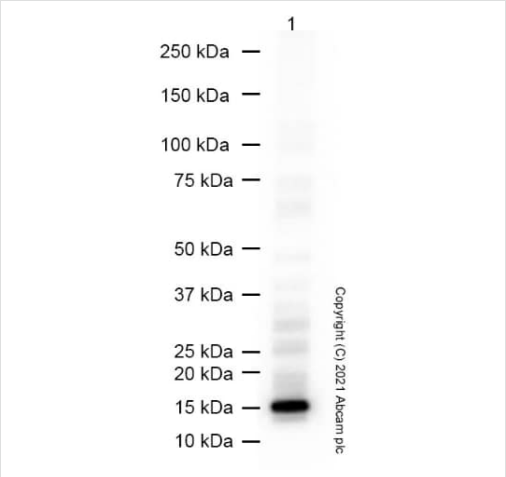
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 antibody [EPR16987] - ChIP Grade (ab176842)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H3 with ab176842 at 1/2000 dilution, followed by Goat anti-rabbit Alexa Fluor® 488 (IgG) (**ab150077**) secondary antibody at 1/400 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue). Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution and **ab150120** (goat anti-mouse AlexaFluor®594 Goat secondary antibody) at 1/500 dilution (red).

The negative controls are as follows;

-ve control 1: ab176842 at 1/2000 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.

-ve control 2: **ab7291** (anti-Tubulin mouse mAb) at 1/500 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/400 dilution.



Western blot - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

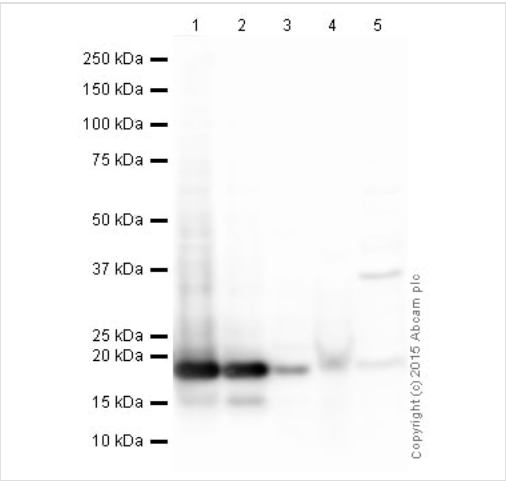
Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) at 1/10000 dilution + HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution

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

Exposure time: 3 seconds

Blocking and dilution buffer: 5% NFDM/TBST



Western blot - Anti-Histone H3 antibody [EPR16987] - ChIP Grade (ab176842)

All lanes : Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842) at 1 µg

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg
Lane 2 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate at 10 µg
Lane 3 : Drosophila embryo nuclear extract (from melanogaster embryos 0-12hr) at 10 µg
Lane 4 : S.cerevisiae (Y190) Whole Cell Lysate at 20 µg
Lane 5 : S.pombe Whole Cell Lysate at 20 µg

Secondary
All lanes : Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/50000 dilution

Developed using the ECL technique.

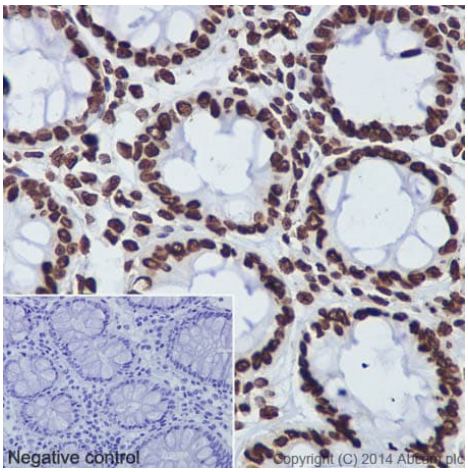
Performed under reducing conditions.

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

Exposure time: 5 seconds

This blot was produced using a 4-12% Bis-tris gel under the MES

buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab176842 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution **ab133406**.

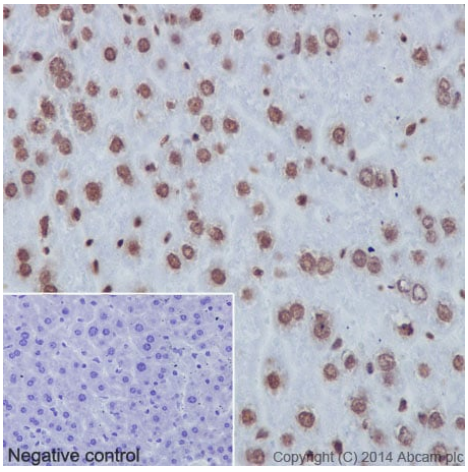


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 antibody [EPR16987] - ChIP Grade (ab176842)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H3 with ab176842 at 1/500 dilution, followed by Goat **Anti-Rabbit HRP** (IgG; H&L) secondary antibody (**ab97051**) at 1/500 dilution. Nuclear staining on glandular epithelium of Human colon tissue is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

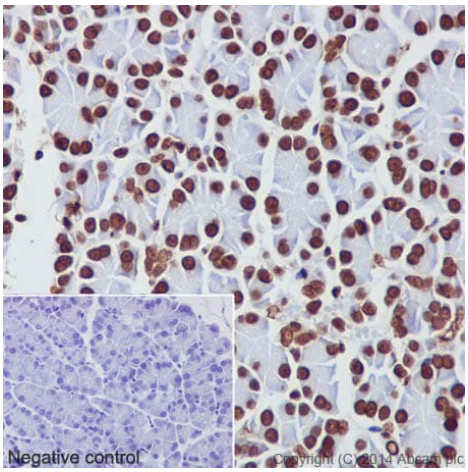


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 antibody [EPR16987] - ChIP Grade (ab176842)

Immunohistochemical analysis of paraffin-embedded Mouse liver tissue labeling Histone H3 with ab176842 at 1/500 dilution, followed by Goat **Anti-Rabbit HRP** (IgG H&L) secondary antibody (**ab97051**) at 1/500 dilution. Nuclear staining on mouse liver tissue is observed. Counter stained with Hematoxylin.

Negative control: Used PBS instead of primary antibody, secondary antibody Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

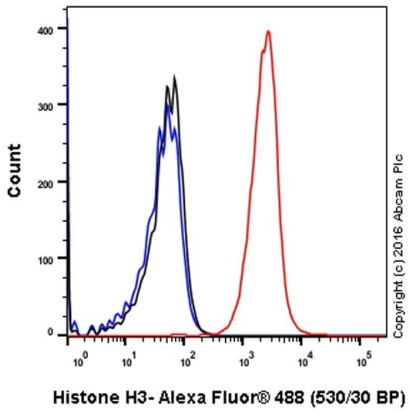


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 antibody [EPR16987] - ChIP Grade (ab176842)

Immunohistochemical analysis of paraffin-embedded Rat pancreas tissue labeling Histone H3 with ab176842 at 1/500 dilution, followed by Goat **Anti-Rabbit HRP** (IgG H&L) secondary antibody (**ab97051**) at 1/500 dilution. Nuclear staining on rat pancreas tissue is observed. Counter stained with Hematoxylin.

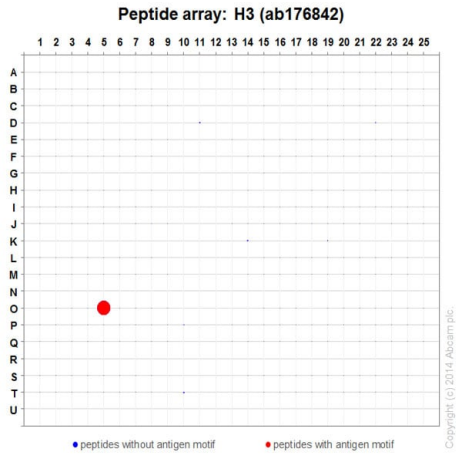
Negative control: Used PBS instead of primary antibody, secondary antibody Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Histone H3 antibody [EPR16987] - Nuclear Marker and ChIP Grade (ab176842)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Histone H3 with purified ab176842 at 1/40 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



Peptide Array - Anti-Histone H3 antibody [EPR16987] - ChIP Grade (ab176842)

ab176842 was tested in Peptide Array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate).
Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.
The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded **here**.

Anti-Histone H3 antibody [EPR16987] - ChIP Grade (ab176842)

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