abcam

Product datasheet

Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - BSA and Azide free ab222481



重组 RabMAb

3 References 11 图像

概述

产品名称 Anti-Histone H3 (tri methyl K27)抗体[EPR18607] - BSA and Azide free

描述 兔单克隆抗体[EPR18607] to Histone H3 (tri methyl K27) - BSA and Azide free

宿主 Rabbit

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

种属反应性 与反应: Mouse. Rat. Human

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. 免疫原

阳性对照 WB: HeLa and NIH/3T3 whole cell lysates; Wild type mouse ES whole cell lysate, IHC-P: Human

> colon, mouse colon and rat kidney tissues. ICC/IF: HeLa cells. ChIP: Chromatin prepared from HeLa cells, Myo-D ChIP primer pair ab269261. ELISA: Histone H3 – unmodified, Histone H3.

常规说明 ab222481 is the carrier-free version of ab192985.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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**.

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性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C. Do Not Freeze.

存储溶液 pH: 7.2

Constituent: PBS

无载体 是

纯**度** Protein A purified

克隆 单克隆

克隆编号 EPR18607

同种型 lgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab222481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		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
PepArr		Use at an assay dependent concentration.
ChIP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ELISA		Use at an assay dependent concentration.
ChIP-sequencing		Use 4µg for 10 ⁷ cells.

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

翻译后修饰

during the process of differentiation.

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.

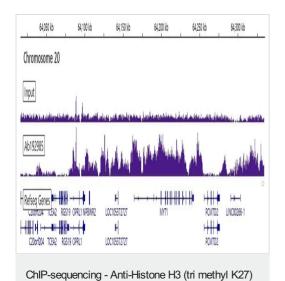
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and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



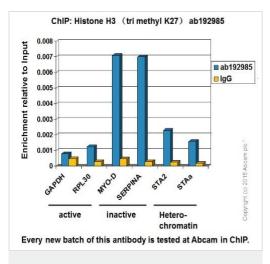
antibody [EPR18607] - BSA and Azide free

(ab222481)

This data was developed using the same antibody clone in a different buffer formulation (<u>ab192985</u>).

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 μ g of Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - ChIP Grade (<u>ab192985</u>). ChIP DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 30 million reads.

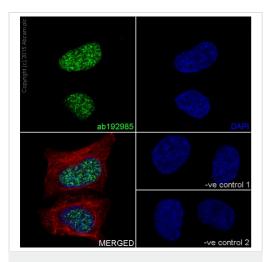
Additional screenshots of mapped reads can be downloaded **here**.



ChIP - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - BSA and Azide free (ab222481)

This ChIP data was generated using the same anti-Histone H3K27me3 antibody clone, EPR18607, in a different buffer formulation (cat# **ab192985**).

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 25µg of chromatin, 2µg of ab192985 (blue), and 20µl of Anti rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] -BSA and Azide free (ab222481)

This ICC/IF data was generated using the same anti-Histone H3K27me3 antibody clone, EPR18607, in a different buffer formulation (cat# <u>ab192985</u>).

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H3 (tri methyl K27) with ab192985 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: <u>ab192985</u> at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (<u>ab150120</u>) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (<u>ab7291</u>) at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution.

Secondary antithody control Contingatively 2015 Abeam pic

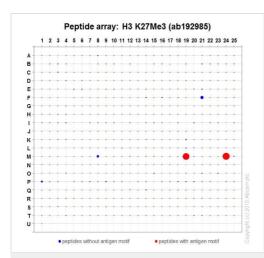
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - BSA and Azide free (ab222481)

Immunohistochemical analysis of paraffin-embedded human colon tissue labeling Histone H3 (tri methyl K27) with <u>ab192985</u> at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on human colon tissue is observed. Counter stained with Hematoxylin.

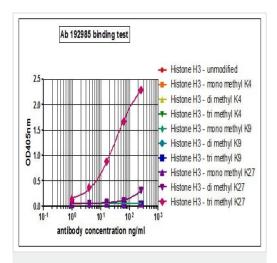
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab192985).

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



Peptide Array - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - BSA and Azide free (ab222481)



ELISA - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - BSA and Azide free (ab222481)

Peptide array analysis was performed using <u>ab192985</u> at a concentration of 0.1ug/ml, followed by Goat Anti-Rabbit lgG, (H+L), Fluo 647nm conjugated secondary antibody at a 1/50000 dilution.

<u>ab192985</u> 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.

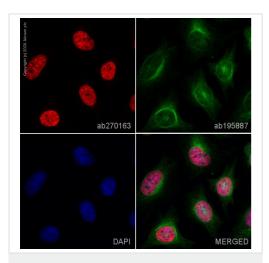
The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded **here**.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab192985</u>).

ELISA analysis was performed on 1ug/ml of antigen using <u>ab192985</u> at a concentration range of 0-0.25ug/ml, followed by Alkaline Phosphatase-conjugates AffiniPure Goat anti-rabbit lgG (H&L) secondary antibody at a 1/2500 dilution.

All batches of <u>ab192985</u> are tested in ELISA against peptides to different Histone H3 modifications. Results show strong binding to Histone H3 - tri methyl K27 immunizing peptide, indicating that this antibody specifically recognizes the Histone H3 - tri methyl K27 modification. Weak binding (14%) was also detected against H3 - di methyl K27 modification.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab192985</u>).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] -BSA and Azide free (ab222481)

ab270162 ab195884

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] -BSA and Azide free (ab222481)

Clone EPR18607 (ab222481) has been successfully conjugated by Abcam. This image was generated using Anti-Histone H3 (tri methyl K27) antibody [EPR18607] (Alexa Fluor® 647). Please refer to ab270163 for protocol details.

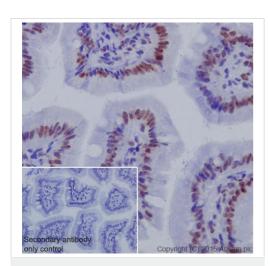
Immunofluorescence staining of Histone H3 (tri methyl K27) in HeLa cells using <u>ab270163</u>. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with <u>ab270163</u> at 1/100 dilution (shown in red) and <u>ab195887</u>, Mouse monoclonal to alpha Tubulin (Alexa Fluor[®] 488), at 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Clone EPR18607 (ab222481) has been successfully conjugated by Abcam. This image was generated using Anti-Histone H3 (tri methyl K27) antibody [EPR18607] (Alexa Fluor® 488). Please refer to ab270162 for protocol details.

Immunofluorescence staining of Histone H3 (tri methyl K27) in HeLa cells using <u>ab270162</u>. The cells were fixed with 4% formaldehyde (10 min), permeabilized with 0.1% 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 <u>ab270162</u> at 1/100 dilution (shown in green) and <u>ab195884</u>, Rat monoclonal to Tubulin (Alexa Fluor[®] 647), at 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



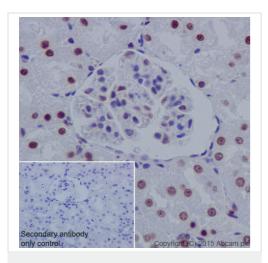
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - BSA and Azide free (ab222481)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Histone H3 (tri methyl K27) with <u>ab192985</u> at 1/500 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on mouse colon tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab192985).

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



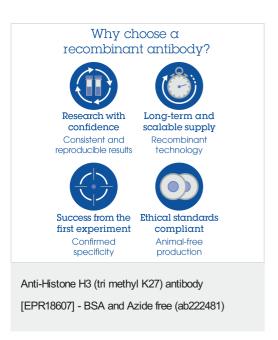
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (tri methyl K27) antibody [EPR18607] - BSA and Azide free (ab222481)

Immunohistochemical analysis of paraffin-embedded rat kidney tissue labeling Histone H3 (tri methyl K27) with <u>ab192985</u> at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Nuclear staining on rat kidney tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab192985</u>).

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



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