abcam

Product datasheet

Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade ab176921

יילעבער RabMAb



★★★★★ 7 Abreviews 7 References

画像数 9

製品の概要

製品名 Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade

製品の詳細 Rabbit monoclonal [EPR16994(2)] to Histone H3 (di methyl K36) - ChIP Grade

由来種 Rabbit

アプリケーション 適用あり: PepArr, ICC/IF, ChIP, IHC-P, WB

種交差性 交差種: Mouse, Rat, Human

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

ポジティブ・コントロール WB: HeLa and NIH/3T3 whole cell lysates. IHC-P: Human stomach, mouse testis and rat spleen

tissues. ICC/IF: HeLa and NIH/3T3 cells. ChIP: Chromatin from HeLa cells.

特記事項 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.

製品の特性

製品の状態 Liquid

保存方法 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.

バッファー Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリモノ モノクローナル クローン名 EPR16994(2)

アイソタイプ ΙgG

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab176921の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
PepArr		Use at an assay dependent concentration.
ICC/IF		1/500.
ChIP		Use 2 µg for 25 µg of chromatin.
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★☆☆ (4)	1/5000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).

ターゲット情報

機能

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.

配列類似性 発生段階

Belongs to the histone H3 family.

翻訳後修飾

Expressed during S phase, then expression strongly decreases as cell division slows down 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.

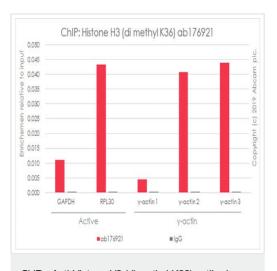
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and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

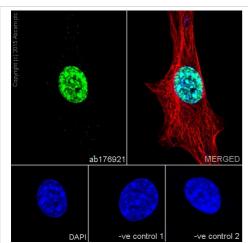
Nucleus. Chromosome.

細胞内局在

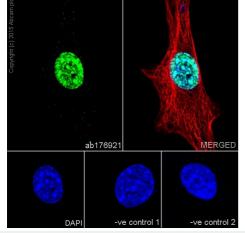
画像



ChIP - Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade (ab176921) 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 ab176921 (red), and 20µl of Anti rabbit IgG sepharose beads. 2µg of rabbit normal IgG was added to the beads control (grey). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)]



ab176921 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).

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling Histone H3 (di methyl K36) with ab176921 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on NIH/3T3 cell line. The

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

-ve control 1: ab176921 at 1/500 dilution, followed by Goat Anti-

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488)

Mouse IgG H&L (Alexa Fluor® 594) (ab150120) secondary

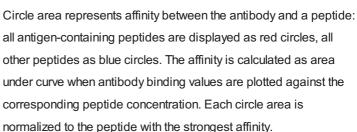
nuclear counter stain is DAPI (blue).

The negative controls are as follows:

antibody at 1/1000 dilution.

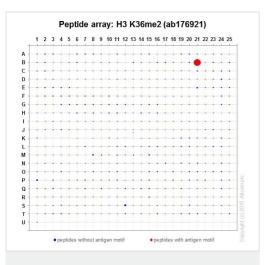
(ab150077) at 1/1000 dilution.

(red).

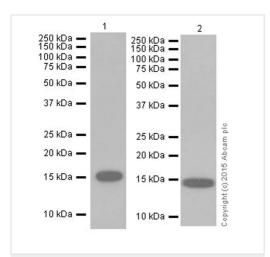


The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded here.





Peptide Array - Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade (ab176921)



Western blot - Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade (ab176921)

All lanes : Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade (ab176921) at 1/5000 dilution

Lane 1: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

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

Lysates/proteins at 10 µg per lane.

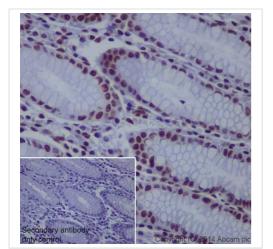
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/1000 dilution

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

Exposure time: 4 seconds

Blocking/Dilution buffer: 5% BSA/TBST.

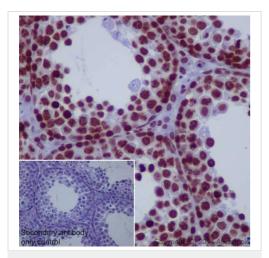


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade (ab176921)

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling Histone H3 (di methyl K36) with ab176921 at 1/250 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on human stomach 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.

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

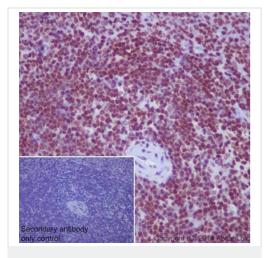


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade (ab176921)

Immunohistochemical analysis of paraffin-embedded mouse testis tissue labeling Histone H3 (di methyl K36) with ab176921 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on mouse testis 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.

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

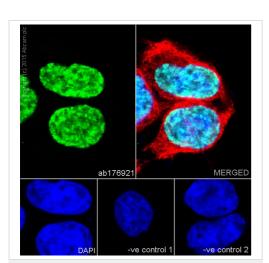


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade (ab176921)

Immunohistochemical analysis of paraffin-embedded rat spleen tissue labeling Histone H3 (di methyl K36) with ab176921 at 1/250 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on rat spleen 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.

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



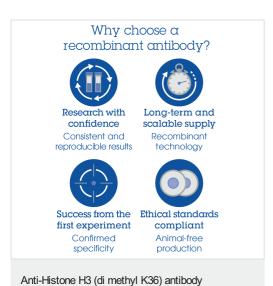
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K36) antibody [EPR16994(2)] - ChIP Grade (ab176921) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Histone H3 (di methyl K36) with ab176921 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain 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: ab176921 at 1/500 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor[®] 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb ($\underline{ab7291}$) at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) ($\underline{ab150077}$) at 1/1000 dilution.



[EPR16994(2)] - ChIP Grade (ab176921)

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 https://www.abcam.co.jp/abpromise or contact our technical team.

Terms and conditions

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