# abcam

## Product datasheet

## Anti-Histone H3 antibody ab18521

★★★★★ 7 Abreviews 66 References 画像数 6

#### 製品の概要

製品名 Anti-Histone H3 antibody

製品の詳細 Rabbit polyclonal to Histone H3

由来種 Rabbit

特異性 ab18521 recognises unmodified and modified forms of histone H3

アプリケーション 適用あり: IP, ICC/IF, WB, IHC-P

種交差性 交差種: Cow, Human, Saccharomyces cerevisiae

交差が予測される動物種: Mouse, Xenopus laevis, Arabidopsis thaliana, Caenorhabditis

elegans, Drosophila melanogaster

免疫原 Synthetic peptide corresponding to Human Histone H3 aa 1-100 (N terminal) conjugated to

keyhole limpet haemocyanin.

Database link: P68431

(Peptide available as ab14949)

ポジティブ・コントロール ICC/IF: HeLa cells. WB: Calf Thymus Histone Preparation Nuclear Lysate. HeLa Histone

Preparation Nuclear Lysate - Colcemid-treated. Histone H3.1 Recombinant Protein. Whole cell lysate prepared from Saccharomyces cerevisiae WT (BY4741, delta hht1 HHT2 and delta hht1

hh2-K9, 14, 18R). IHC-P: Human testis tissue. IP: HeLa whole cell extract.

**特記事項**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

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 or -

80°C. Avoid freeze / thaw cycle.

**バッファー** pH: 7.40

Preservative: 0.02% Sodium azide

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#### 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.

精製度 Immunogen affinity purified

**ポリ/モノ** ポリクローナル

アイソタイプ lgG

#### アプリケーション

**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ab18521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
IP		Use a concentration of 5 µg/ml.
ICC/IF	*** <u>*</u>	Use a concentration of 1 - 5 μg/ml.
WB	<b>★★★★</b> ★ ★ (5)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
IHC-P		Use a concentration of 1 $\mu$ g/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

## 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** Belongs to the histone H3 family.

**発生段階** Expressed during S phase, then expression strongly decreases as cell division slows down

during the process of differentiation.

翻訳後修飾 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.

 $\label{lem:asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation.}$ 

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.

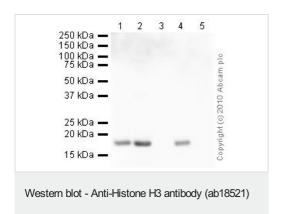
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and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



All lanes: Anti-Histone H3 antibody (ab18521) at 1 µg/ml

**Lane 1 :** Calf Thymus Histone Preparation Nuclear Lysate at  $0.5~\mu g$ 

Lane 2: HeLa Histone Preparation Nuclear Lysate - Colcemid-

treated at 2.5 µg

Lane 3: Histone H2A Recombinant Protein at 0.1 µg

Lane 4: Histone H3.1 Recombinant Protein at 0.1 µg

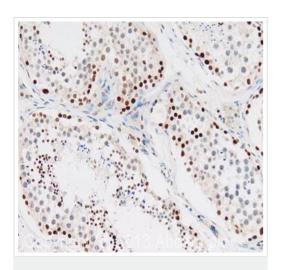
Lane 5: Histone H4 Recombinant Protein at 0.1 µg

#### Secondary

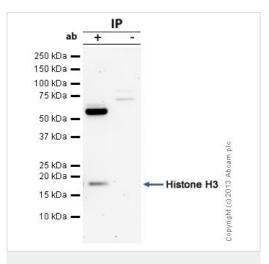
 $\begin{tabular}{ll} \textbf{All lanes:} Goat polyclonal to Rabbit lgG - H\&L - Pre-Adsorbed (HRP) at 1/3000 dilution \end{tabular}$ 

Performed under reducing conditions.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 antibody (ab18521)



Immunoprecipitation - Anti-Histone H3 antibody (ab18521)

IHC image of Histone H3 staining in human testis formalin fixed paraffin embedded tissue section, performed on a Leica Bond TM system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab18521, 1µg/ml, for 15 mins 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.

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.

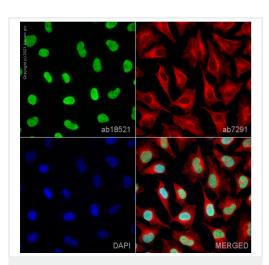
Histone H3 was immunoprecipitated using 0.5mg Hela whole cell extract, 5µg of Rabbit polyclonal to Histone H3 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab18521.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

Band: 17kDa; Histone H3



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 antibody (ab18521)

1 2 3
(kDa)
70 55 40 36 25 15 -

Western blot - Anti-Histone H3 antibody (ab18521)

This image was kindly supplied by Dr Tobias Eisenberg by Abreview

ab18521 staining Histone H3 in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 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 ab18521 at 1 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), preadsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

**All lanes:** Anti-Histone H3 antibody (ab18521) at 1/250 dilution

**Lane 1 :** Whole cell lysate prepared from *Saccharomyces cerevisiae* WT (BY4741).

**Lane 2**: Whole cell lysate prepared from *Saccharomyces cerevisiae* delta hht1 HHT2.

**Lane 3**: Whole cell lysate prepared from *Saccharomyces cerevisiae* delta hht1 hh2-K9, 14, 18R.

Lysates/proteins at 500000 cells per lane.

#### **Secondary**

All lanes: HRP conjugated goat anti-rabbit IgG at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 10 minutes

Blocking performed using 3% BSA for 16 hours at 4°C. Lysates prepared using 5% Trichloroethane.

All lanes: Anti-Histone H3 antibody (ab31602) at 1 µg/ml

Lane 1: Calf thymus histone lysate

Lane 2 : Calf thymus histone lysate with Human Histone H3 peptide (ab14949) at 1 µg/ml

**Lane 3 :** Calf thymus histone lysate with Human Histone H3 (mono methyl K4) peptide (<u>ab1340</u>) at 1  $\mu$ g/ml

**Lane 4**: Calf thymus histone lysate with Human Histone H3 (di methyl K4) peptide (<u>ab7768</u>) at 1 μg/ml

**Lane 5 :** Calf thymus histone lysate with Human Histone H3 (tri methyl K4) peptide (**ab1342**) at 1 μg/ml

Lysates/proteins at 20 µg per lane.

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

ab18521 recognises histone H3 as well as modified histone H3 at approximately 15 kDa(lane1). This is demonstrated by the blocking using the immunising unmodified histone H3 peptide (lane2), histone H3 mono methyl K4 (lane3), histone H3 di methyl K4 (lane4) and histone H3 tri methyl K4 (lane5).

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25 kDa -20 kDa -

15 kDa -

10 kDa -

Western blot - Anti-Histone H3 antibody (ab18521)

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