# abcam

### Product datasheet

## Anti-Histone H3.3 (phospho S31) antibody [EPR1873] ab92628



重组 RabMAb

★★★★★ 4 Abreviews 13 References 8 图像

概述

产品名称 Anti-Histone H3.3 (phospho S31)抗体[EPR1873]

描述 兔单克隆抗体[EPR1873] to Histone H3.3 (phospho S31)

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), Dot blot, WB, IHC-P, ICC/IF

不适用干: IP

种属反应性 与反应: Human

预测可用于: Mouse, Rat 🗥

免疫原 Synthetic peptide within Human Histone H3.3 aa 1-100 (phospho S31). The exact sequence is

proprietary.

Database link: P84243

阳性对照 Untreated Hela cells and HeLa cell lysate treated with Calyculin A and/or nocodazole. Human

cervical carcinoma

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 -20°C. Stable for 12 months at -20°C.

存储溶液

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

纯度 Protein A purified

克隆 单克隆

**克隆编号** EPR1873

**同种型** IgG

#### 应用

The Abpromise guarantee Abpromise™承诺保证使用ab92628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
Flow Cyt (Intra)		Use at an assay dependent concentration.
Dot blot		1/1000.
WB	★★★☆☆(3)	1/1000 - 1/5000. Predicted molecular weight: 15 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	**** <u>(1)</u>	1/100 - 1/250.

应用说明

Is unsuitable for IP.

#### 靶标

#### 功能

Variant histone H3 which replaces conventional H3 in a wide range of nucleosomes in active genes. Constitutes the predominant form of histone H3 in non-dividing cells and is incorporated into chromatin independently of DNA synthesis. Deposited at sites of nucleosomal displacement throughout transcribed genes, suggesting that it represents an epigenetic imprint of transcriptionally active chromatin. 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

发展阶段

翻译后修饰

Belongs to the histone H3 family.

Expressed throughout the cell cycle independently of DNA synthesis.

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.

Specifically enriched in modifications associated with active chromatin such as methylation at Lys-5 (H3K4me), Lys-37 and Lys-80. 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), which are linked to gene repression, are underrepresented. 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.

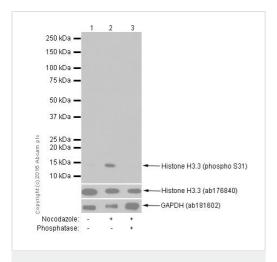
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and 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. Phosphorylation on Ser-32 (H3S31ph) is specific to regions bordering centromeres in metaphase chromosomes.

Ubiquitinated. Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination.

Nucleus. Chromosome.

细胞定位

图片



Western blot - Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628)

**All lanes :** Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628) at 1/1000 dilution

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

Lane 2: Whole cell lysate from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with 100ng/ml of nocodazole for 18 hours

**Lane 3**: Whole cell lysate from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with 100ng/ml of nocodazole for 18 hours. Membrane incubated with phosphatase

Lysates/proteins at 15 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 15 kDa

Exposure time: 15 seconds

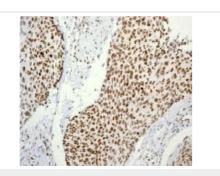
Blocking/dilution buffer: 2% BSA/TBST

Ab92628 Anti-Histone H3.3 (phospho S31) Ab176840 Anti-Histone H3.3 (phospho S31) Ab176840 Ab178840-DAPI-tubulin Ab176840 Ab178840-DAPI-tubulin Ab176840 Ab176840-DAPI-tubulin Ab176840-DAPI-tub

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628) Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% tritonX-100 HeLa (Human epithelial cell line from cervix adenocarcinoma) cells, LP treated and non-treated, labeling anti-Histone H3.3 (phospho S31) with ab92628 at 1/500 dilution followed by Goat anti-Rabbit secondary IgG Alexa Fluor<sup>®</sup> 488 (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on M phase of HeLa cells, then the signal decreased after LP treatment.

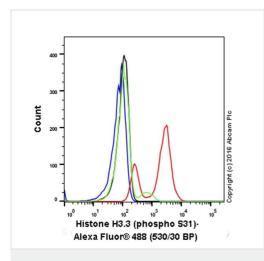
For the pan antibody, there was no great difference after LP treatment. The data showed mostly nuclear staining.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628)

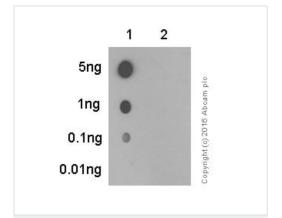
Paraffin-embedded human cervical carcinoma labelled with ab92628 at 1/100 dilution.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628)

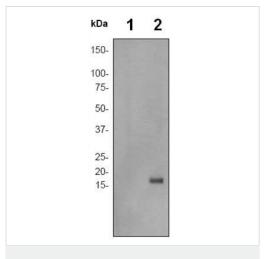
Intracellular Flow Cytometry analysis of Hela (human cervix adenocarcinoma) cells treated with 100 ng/ml Nocodazole for 18 hours, labeling Histone H3.3 with purified ab92628 at 1/150 dilution (10µg/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti-rabbit lgG (Alexa Fluorr<sup>®</sup> 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control. Untreated control - Hela (human cervix adenocarcinoma) cells untreated with 100 ng/ml Nocodazole for 18 hours (Green).



Dot Blot - Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628)

Dot Blot analysis of **Lane 1**: Histone H3.3 (pS31) phospho peptide and **Lane 2**: Histone H3.3 non-phospho peptide labeling Histone H3.3 (phospho S31) with ab92628 at 1/1000 dilution. 5% NFDM/TBST was used as the diluting and blocking buffer.

<u>ab97051</u> Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated was used as the secondary antibody at 1/100000 dilution. Exposure time: 10 seconds.



Western blot - Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628)

**All lanes :** Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628) at 1/1000 dilution

Lane 1: HeLa cell lysate untreated

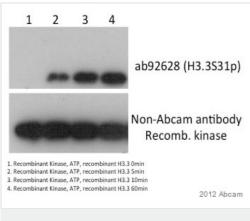
Lane 2: HeLa cell lysate treated with Calyculin A

Lysates/proteins at 10 µg per lane.

#### **Secondary**

All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 15 kDa

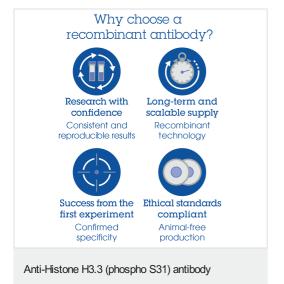


Western blot - Anti-Histone H3.3 (phospho S31) antibody [EPR1873] (ab92628)

This image is courtesy of an anonymous Abreview

Representative western blot detecting Histone H3.3 (phospho S31) using ab92628 at 1/3000 dilution.

Recombinant Histone H3.3 protein was treated with recombinant kinase and 200  $\mu$ M ATP for the indicated times. 0.2  $\mu$ g of protein was loaded to each lane and phosphorylated Histone H3.3 was detected using ab92628. An HRP-conjugated goat anti-rabbit polyclonal (1/20000) was used as the secondary antibody



[EPR1873] (ab92628)

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