abcam

Product datasheet

Anti-Histone H3 (phospho S28) antibody ab5169

★★★★★ 3 Abreviews 16 References 5 图像

概述

产品名称	Anti-Histone H3 (phospho S28)**抗体**
描述	**兔多克隆抗体**to Histone H3 (phospho S28)
宿主	Rabbit
特异性	This antibody is specific for Histone H3 phosphorylated at residue Ser 28 and does not recognise the unmodified residue or another phosphorylated residue (Ser 10) on the same histone.
经测试应用	**适用于:** WB, PepArr, ICC/IF
种属反应性	**与反应:** Human, Recombinant fragment **预测可用于:** Drosophila melanogaster
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
常规说明	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

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 or -80°C. Avoid freeze / thaw cycle.
存储溶液	pH: 7.40 Preservative: 0.02% Sodium azide 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.
纯度	Immunogen affinity purified
克隆	**多克隆**

同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab5169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
WB	★★★★★ (2)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).
PepArr		Use a concentration of 0.02 - 0.002 µg/ml.
ICC/IF		1/5000.

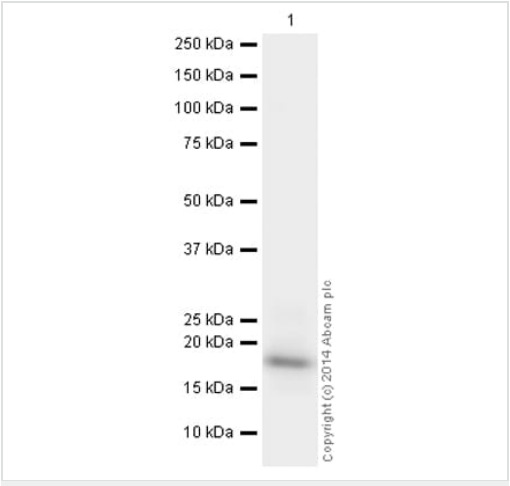
靶标

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



Western blot - Anti-Histone H3 (phospho S28) antibody (ab5169)

Anti-Histone H3 (phospho S28) antibody (ab5169) at 1 µg/ml +
Hela Whole Cell Lysate - Colcemid Treated at 2.5 µg

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

Developed using the ECL technique.

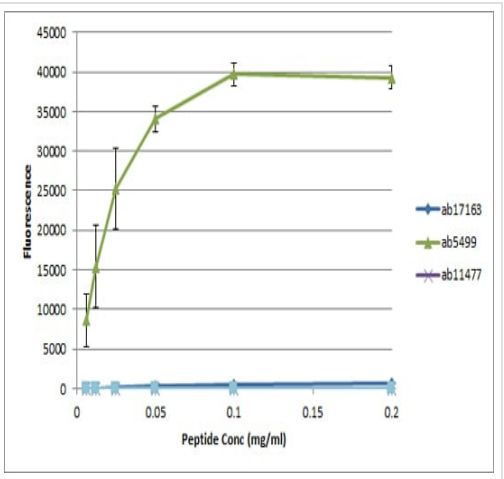
Performed under reducing conditions.

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

Exposure time: 30 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 ab5169 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution **ab133406**.



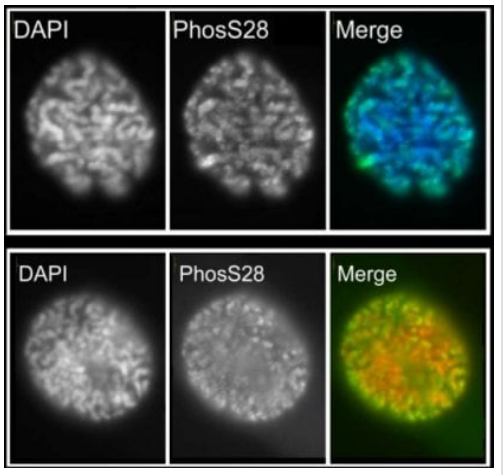
Peptide Array - Anti-Histone H3 (phospho S28) antibody (ab5169)

All batches of ab5169 are tested in Peptide Array against peptides to different Histone H3 modifications. Six dilutions of each peptide are printed on to the Peptide Array in triplicate and results are averaged before being plotted on to a graph. Results show strong binding to Histone H3 - phospho S28 peptide (**ab5499**), indicating that this antibody specifically recognises the Histone H3 - phospho S28 modification.

ab17163 - Histone H3 unmodified

ab5499 - Histone H3 - phospho S28

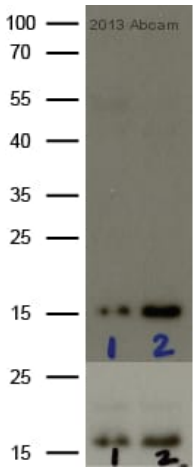
ab11477 - Histone H3 - phospho S10



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S28) antibody (ab5169)

The image was submitted as part of a review by Krik McManus, University of British Columbia

Indian Muntjac (top panel) and HeLa cells (bottom panel) immunofluorescently labelled with ab5169 (green) at a working dilution of 1/5000. The DNA is counterstained with DAPI and is shown in blue in the top panel and red in the bottom panel. This antibody gives a characteristic staining pattern for Histone H3 (phospho S28) whereby the signal increases in intensity during late G2 and continues to increase until metaphase. Upon entry into anaphase the signal begins to decrease until reaching basal levels by early G1. 100x magnification.



Western blot - Anti-Histone H3 (phospho S28) antibody (ab5169)

Image courtesy of Richelle Sopko, Harvard University, U.S.A

All lanes : Anti-Histone H3 (phospho S28) antibody (ab5169) at 1/1000 dilution

Lane 1 : Wild type 0-4 hour old fruit fly embryos.
Lane 2 : 0-4 hour old fruit fly embryos expressing wee RNAi

Secondary
All lanes : Donkey anti-rabbit IgG, Horseradish Peroxidase-L at 1/10000 dilution

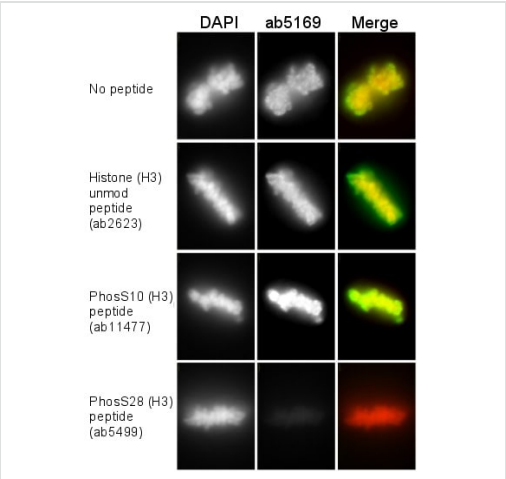
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa

Exposure time: 10 minutes

0-4hr old wee shRNA embryos (lane 2) should display elevated phH3Ser28 levels relative to 0-4hr old EGFP shRNA embryos (lane 1). Blocked with 10% BSA.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (phospho S28) antibody (ab5169)

This image was submitted as part of a review by Kirk McManus, University of British Columbia

In situ peptide competition was perfomed on paraformaldehyde-fixed HeLa cells. Four 25µl aliquots were made, to which 7.5µg (1.5µl) of no peptide, H3 unmodified peptide (**ab2623**), H3 phospho S10 peptide (**ab11477**) or H3 phospho S28 peptide (**ab5499**) was added, mixed by vortexing and incubated for 1 hour at room temperature. Cells on glass coverslips were fixed with 4% paraformaldehyde (10min) and gently washed twice with PBS, then permeabilized with 0.5% Triton X-100 in PBS (10min) and gently washed three times with PBS. The cells were immunofluorescently labeled with either the peptide-competed antibody or the control antibody (i.e. no peptide) for 30min at room temperature, washed briefly with PBS containing 0.1% Triton X-100 (1 min) and twice with PBS. The cells were then incubated with an appropriate dilution of a secondary antibody at room temperature for 30min, rinsed as above and mounted using a 90% glycerol in PBS mount