abcam

Product datasheet

Anti-Histone H3 (phospho S28) antibody [HTA28] ab10543

12 Abreviews 106 References 6 Images

Overview

Product name	Anti-Histone H3 (phospho S28) antibody [HTA28]
Description	Rat monoclonal [HTA28] to Histone H3 (phospho S28)
Host species	Rat
Tested applications	**Suitable for:** WB, ICC
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Hamster, Cow
Immunogen	Synthetic peptide corresponding to Human Histone H3 aa 23-35 (phospho S28) conjugated to Keyhole Limpet Haemocyanin (KLH). Sequence: KAARKSA PATGGV Database link: **P68431** Run BLAST with Run BLAST with
Positive control	WB: HeLa (treated with Nocodazole) whole cell extract. ICC: HeLa cells.
General notes	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

Properties

Form	Liquid
Storage instructions	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.
Storage buffer	pH: 7.40 Preservative: 0.097% Sodium azide Constituents: 0.0268% PBS, 1% BSA

Purity	Immunogen affinity purified
Purification notes	Purified from culture supernatant of hybridoma cells grown in a bioreactor
Clonality	Monoclonal
Clone number	HTA28
Isotype	IgG2a

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab10543 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★☆☆ (1)	Use a concentration of 0.5 - 2 µg/ml. Detects a band of approximately 15 kDa. We advise you to centrifuge this product vial before use.
ICC	★★★★★ (2)	Use a concentration of 5 µg/ml.

Target

Function 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.

Sequence similarities Belongs to the histone H3 family.

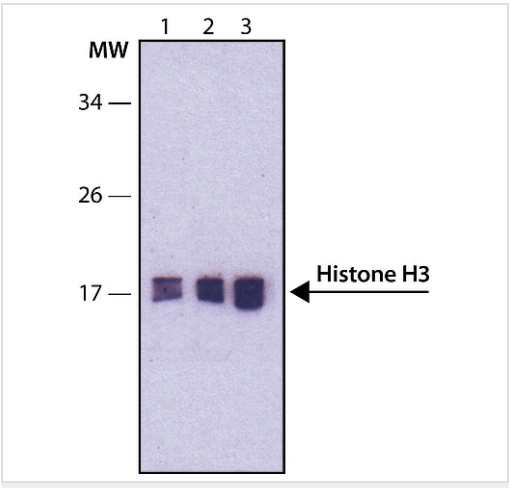
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

Post-translational modifications 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.

Cellular localization Nucleus. Chromosome.

Images



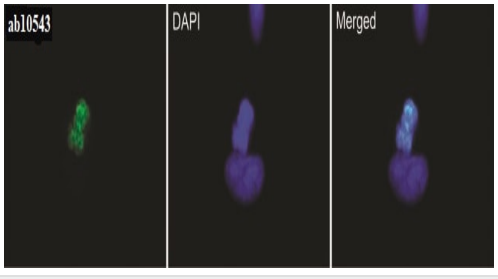
Western blot - Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543)

Lane 1 : Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543) at 0.5 µg/ml
Lane 2 : Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543) at 1 µg/ml
Lane 3 : Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543) at 2 µg/ml

All lanes : HeLa (treated with Nocodazole) whole cell extract

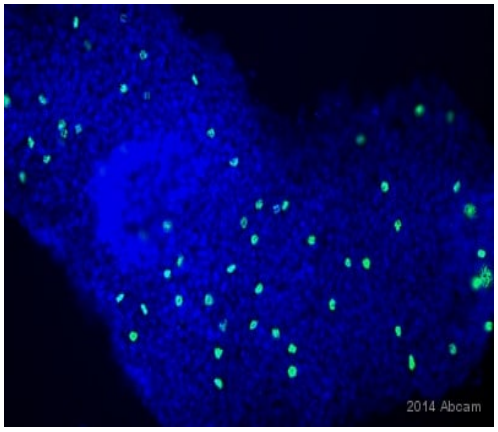
Secondary
All lanes : Goat Anti-Rat IgG-peroxidase conjugate

Developed using the ECL technique.



Immunocytochemistry - Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543)

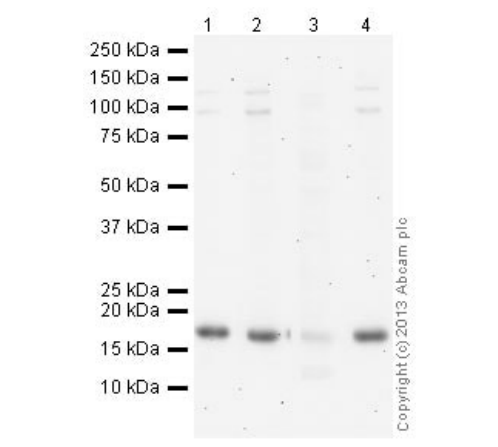
Immunocytochemical analysis of HeLa cells fixed and permeabilized with methanol followed by methanol:acetone. Histone H3 (phospho S28) was labeled with ab10543 at 5 µg/mL in ICC/IF (Green). The secondary antobody was a Goat Anti-Rat IgG-FITC conjugate. The nuclear counerstain was DAPI (Blue).



Immunocytochemistry - Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543)

This image is courtesy of an anonymous Abreview

ab10543 staining Histone H3 (phospho S28) in Human neural progenitor cells from iPS cell line by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton and blocked with 1% serum, 0.1% BSA in PBS for 30 minutes at room temperature. Samples were incubated with primary antibody (2ug/ml in blocking buffer) for 16 hours at 4°C. An Alexa Fluor® 488-conjugated Goat anti-rat IgG2a polyclonal was used as the secondary antibody (1/500). Total cells were stained using DAPI (blue)



Western blot - Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543)

All lanes : Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543) at 1 µg/ml

Lane 1 : HeLa Histone Preparation Nuclear Lysate - Colcemid-treated
Lane 2 : HeLa Histone Preparation Nuclear Lysate - Colcemid-treated with Human Histone H3 (unmodified) peptide (**ab2623**) at 0.5 µg/ml
Lane 3 : HeLa Histone Preparation Nuclear Lysate - Colcemid-treated with Human Histone H3 (phospho S28) peptide (**ab5499**) at 0.5 µg/ml
Lane 4 : HeLa Histone Preparation Nuclear Lysate - Colcemid-treated with Human Histone H3 (phospho S10) peptide (**ab11477**) at 0.5 µg/ml

Lysates/proteins at 2.5 µg per lane.

Secondary

All lanes : Peroxidase Conjugated AffiniPure Rabbit Anti-Rat IgG (H+L) at 1/10000 dilution

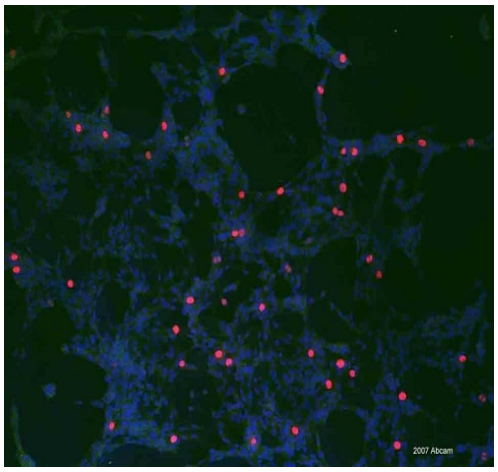
Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 17 kDa

Exposure time: 20 minutes

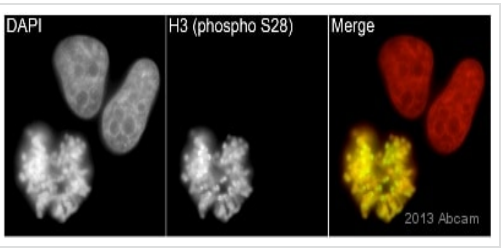
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin before being incubated with ab10543 overnight at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution.



Immunocytochemistry - Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543)

This image is courtesy of an anonymous Abreview

ab10543 staining Histone H3 (phospho S28) in Mouse neural stem cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with paraformaldehyde and blocked with 4% serum for 1 hour at 20°C. Samples were incubated with primary antibody (1/500 in PBS + 4% serum) for 16 hours at 4°C. An Alexa Fluor® 546-conjugated anti-rat polyclonal was used as the secondary antibody (1/500). DAPI is stained blue



Immunocytochemistry - Anti-Histone H3 (phospho S28) antibody [HTA28] (ab10543)

This image is courtesy of an Abreview submitted by Dr Kirk McManus

ab10543 staining HeLa cells by ICC/IF. The cells were fixed with paraformaldehyde and permeabilized with 0.5% Triton X100 in PBS. The cells were then stained with ab10543 at 1/1000 in PBS for 1h at 22°C. A goat **anti-rat Alexa Fluro 488** (**ab150157**) at 1/200 was used as the secondary antibody. Nuclei are stained in red with DAPI. The antibody produces the expected mitotic-associated staining pattern and is extremely strong. MeOH fixed samples were also evaluated and produced a similar, strong staining pattern in mitotic cells.

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