abcam

Product datasheet

Anti-Histone H3 (phospho T32) antibody ab4076

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Overview

Product name Anti-Histone H3 (phospho T32) antibody

Description Rabbit polyclonal to Histone H3 (phospho T32)

Host species Rabbit

Specificity From Jan 2024, QC testing of replenishment batches of this polyclonal changed. All tested and

expected application and reactive species combinations are still covered by our Abcam product promise. However, we no longer test all applications. For more information on a specific batch,

please contact our Scientific Support who will be happy to help.

Tested applications
Suitable for: IHC-P, WB
Species reactivity
Reacts with: Cow, Human

Predicted to work with: Mouse, Rat, Chicken, Tetrahymena, Xenopus laevis, Drosophila

melanogaster, Zebrafish
Does not react with: Saccharomyces cerevisiae

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

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: 0.02% Sodium azide

Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

scientific support team who will be happy to help.

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Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab4076 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
IHC-P		Use a concentration of 4 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa.

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

Developmental stage

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

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).

Belongs to the histone H3 family.

during the process of differentiation.

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.

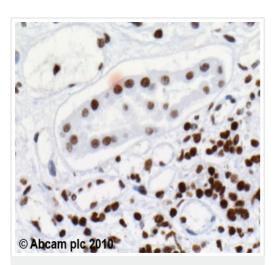
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and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

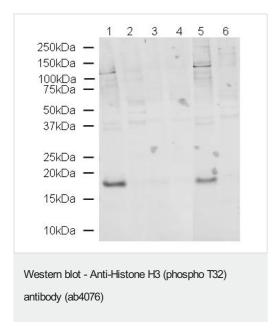
Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (phospho T32) antibody (ab4076)

ab4076 (4µg/ml) staining Histone H3 in human placenta using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear staining.

Sections were rehydrated and antigen retrieved with the Dako 3-in-1 AR buffer citrate pH 6.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



All lanes : Anti-Histone H3 (phospho T32) antibody (ab4076) at 1 $\mu g/ml$

Lane 1: Colcemid treated histone calf thymus lysate

Lane 2: Untreated histone calf thymus lysate

Lane 3 : Colcemid treated histone calf thymus lysate with Human Histone H3 (phospho T32) peptide (**ab14799**) at 1 μg/ml

Lane 4: Untreated calf thymus histone lysate with Human Histone H3 (phospho T32) peptide (ab14799) at 1 µg/ml

Lane 5 : Colcemid treated calf thymus histone lysate with Human Histone H3 (unmodified) peptide (ab2623) at 1 µg/ml

Lane 6 : Untreated calf thymus histone lysate with Human Histone H3 (unmodified) peptide (<u>ab2623</u>) at 1 μ g/ml

Lysates/proteins at 20 µg per lane.

ab4076 recognises a band at 17 kDa, corresponding to Histone H3, in colcemid treated lysates indicating that it is a phosphospecific antibody. ab4076 is specifically blocked using the immunizing peptide (**ab14799**), but not the unmodified control peptide (**ab2623**).

This indicates that <u>ab4079</u> is specifically recognising
Phosphorylated T32 of Histone H3. ab4076 specifically recognises
the phosphorylated form of Histone H3

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