abcam

Product datasheet

Anti-Histone H3 (citrulline R26) antibody [EPR20606] ab212082



Overview

Product name Anti-Histone H3 (citrulline R26) antibody [EPR20606]

Description Rabbit monoclonal [EPR20606] to Histone H3 (citrulline R26)

Host species Rabbit

Tested applications Suitable for: WB, Dot blot, ELISA

Species reactivity Reacts with: Mouse, Human

Immunogen This product was produced with the following immunogens:

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

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Positive control WB: HEK-293T transfected with GFP-tagged PADI4 (WT) whole cell lysate treated with CaCl2

and lonomycin; NIH/3T3 transfected with GFP-tagged PADI4 (WT) whole cell lysate treated with

CaCl2.

General notesThis 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**.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), PBS

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Purity Protein A purified

Clonality Monoclonal
Clone number EPR20606

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab212082 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		1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
Dot blot		1/1000.
ELISA		Use a concentration of 0 - 1 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 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.

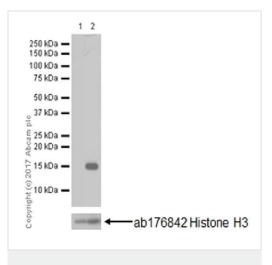
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and 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 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 (citrulline R26) antibody [EPR20606] (ab212082)

All lanes : Anti-Histone H3 (citrulline R26) antibody [EPR20606] (ab212082) at 1/1000 dilution

Lane 1 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) transfected with an empty vector (control) then treated with 10 mM CaCl2 and 10 μ M lonomycin for 2 hours, whole cell lysate

Lane 2 : HEK-293T transfected with a GFP-tagged PAD4 expression vector then treated with 10 mM CaCl2 and 10 μ M lonomycin for 2 hours, whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/100000 dilution

Developed using the ECL technique.

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

Exposure time: 5 seconds

Histone H3R26 is citrullinated by PAD4, $CaCl_2$ is used as a cofactor according to the literature (PMID: 16567635). Ionomycin is used to improve the modification by PAD4 according to the literature (PMID: 26360112).

Blocking/Dilution buffer: 5% BSA/TBST.

Dot blot analysis of Histone H3 (citrulline R26) labeled with ab212082 at 1/1000 dilution.

Lane 1: Histone H3 (citrulline R26) peptide (aa25-36)

Lane 2: Histone H3 (Citrulline R17) peptide (aa1-21)

Lane 3: Histone H3 (Citrulline R8) peptide (aa1-21)

Lane 4: Histone H3 (Citrulline R2) peptide (aa1-21)

Lane 5: Histone H3 peptide (aa1-21)

Lane 6: Histone H3 peptide (aa19-36)

Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution was used as secondary antibody.

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: 30 seconds.

All lanes : Anti-Histone H3 (citrulline R26) antibody [EPR20606] (ab212082) at 1/1000 dilution

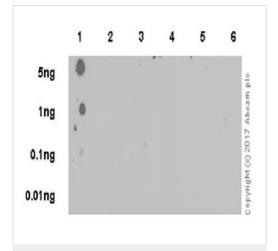
Lane 1: NIH/3T3 (mouse embryonic fibroblast cell line) transfected with a GFP-tagged empty vector (control) then treated with 10 mM CaCl2 for 2 hours, whole cell lysate

Lane 2: NIH/3T3 transfected with a GFP-tagged PADI4 expression vector treated with 10 mM CaCl2 for 2 hours, whole cell lysate

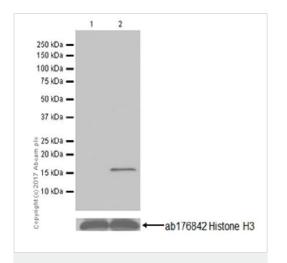
Lysates/proteins at 10 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at



Dot Blot - Anti-Histone H3 (citrulline R26) antibody [EPR20606] (ab212082)



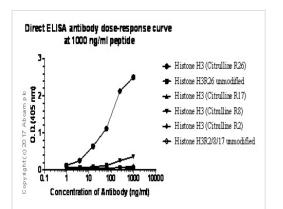
Western blot - Anti-Histone H3 (citrulline R26) antibody [EPR20606] (ab212082)

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

Exposure time: 3 minutes

Histone H3R26 is citrullinated by PAD4, $CaCl_2$ is used as a cofactor according to the literature (PMID: 16567635). lonomycin is used to improve the modification by PAD4 according to the literature (PMID: 26360112).

Blocking/Dilution buffer: 5% BSA/TBST.



ELISA - Anti-Histone H3 (citrulline R26) antibody [EPR20606] (ab212082)

ELISA using ab212082 labeling Histone H3 (Citrulline R26), Histone H3R26 unmodified, Histone H3 (Citrulline R17), Histone H3 (Citrulline R8), Histone H3 (Citrulline R2) and Histone H3R2/8/17 unmodified at 1 μ g/ml. ab212082 was used at a range between 0 - 1 μ g/ml.

Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit lgG(H+L) was used as secondary antibody at 1/2500 dilution. ab212082 cross binds H3R8Cit at 14%.



[EPR20606] (ab212082)

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