abcam

Product datasheet

Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] ab281584

Recombinant RabMAb

3 Abreviews 8 Images

Overview

Product name	Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001]
Description	Rabbit recombinant multiclonal [RM1001] to Histone H3 (citrulline R2 + R8 + R17)
Host species	Rabbit
Tested applications	**Suitable for:** IP, PepArr, ICC/IF, Flow Cyt (Intra), WB, Dot blot **Unsuitable for:** ChIP or IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	**This product was produced with the following immunogens:** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK-293T, NIH/3T3 cells transfected with PADI4 expression vector containing a myc-His-tag® treated with 10 mM CaCl2 and 10 µM Ionomycin for 4 h, C6 cells transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM CaCl2 and 10 µM Ionomycin for 2 h. IP: HEK-293T cells. ICC/IF: 293T cells. Flow Cyt (Intra): 293T cells transfected with myc-tagged PADI4 construct, then treated with 10mM CaCl2 and 10uM Ionomycin for 4h.
General notes	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: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Recombinant Multiclonal

Clone number	RM1001
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab281584 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
IP		1/30.
PepArr		Use at an assay dependent concentration.
ICC/IF		1/2000.
Flow Cyt (Intra)		1/500.
WB		1/1000. Predicted molecular weight: 15 kDa.
Dot blot		1/1000.

Application notes Is unsuitable for ChIP or IHC-P.

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.

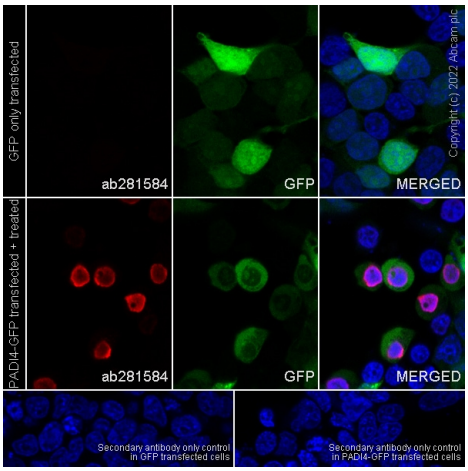
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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.

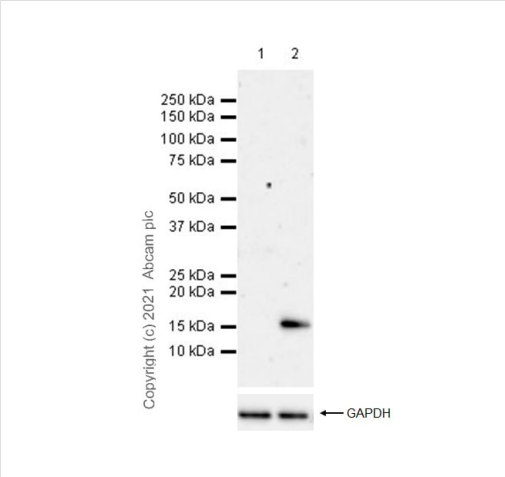
Cellular localization Nucleus. Chromosome.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 100% methanol permeabilized 293T (human embryonic kidney epithelial cell) cells labelling Histone H3 (citrulline R2 + R8 + R17) with primary antibody anti-Histone H3 (citrulline R2 + R8 + R17) (ab281584) at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) (**ab150080**) secondary antibody at 1/1000 dilution. Confocal image showing nuclear staining on 293T cells transfected with a human PADI4 expression vector containing a GFP tag, then treated with 10 mM CaCl2 and 10 µM ionomycin for 2 hours. The nuclear counter stain is DAPI (blue).



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

All lanes : Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584) at 1/1000 dilution

Lane 1 : Untreated C6 (rat glial tumor glial cell) transfected with PADI4 expression vector containing a GFP-tag, whole cell lysate
Lane 2 : C6 transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM Ionomycin for 2 hours, whole cell lysate

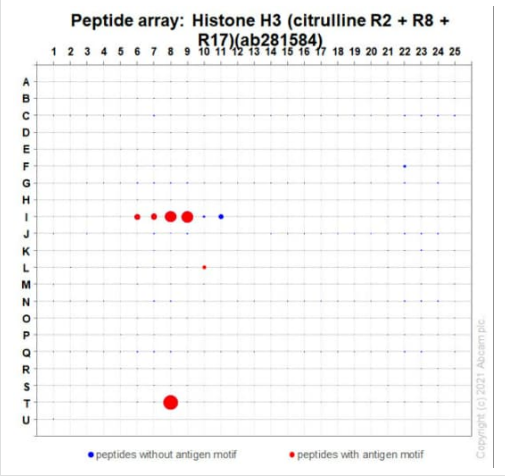
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 15 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 180 seconds

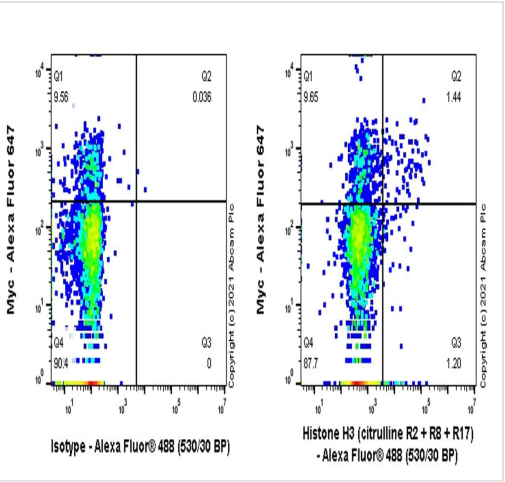


Peptide Array - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

Peptide array analysis of ab281584 at (0.1ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Fluo 647nm conjugated at 1:50,000 dilution.

All batches of ab281584 are tested in Peptide Array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate).

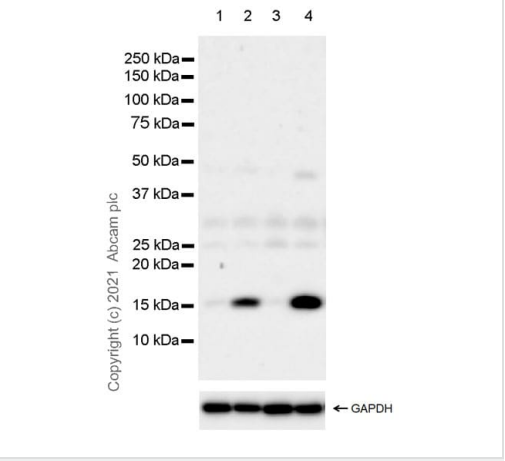
Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.
The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded **here**



Flow Cytometry (Intracellular) - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

Intracellular Flow Cytometry analysis of 293T (human embryonic kidney epithelial cell) transfected with myc-tagged PADI4 construct, then treated with 10mM CaCl2 and 10uM Ionomycin for 4h labeling Histone H3 (citrulline R2 + R8 + R17) with ab281584 at 1/500 dilution. Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as a secondary antibody.

Isotype control : Rabbit monoclonal IgG (**ab172730**)/left.



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

All lanes : Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584) at 1/1000 dilution

Lane 1 : Untreated HEK-293T (human embryonic kidney) transfected with PADI4 expression vector containing a myc-His-tag®, whole cell lysate
Lane 2 : HEK-293T transfected with PADI4 expression vector containing a myc-His-tag® treated with 10 mM calcium chloride and 10 µM Ionomycin for 4 hours, whole cell lysate
Lane 3 : Untreated NIH/3T3 (mouse embryonic fibroblast) transfected with PADI4 expression vector containing a myc-His-tag®, whole cell lysate
Lane 4 : NIH/3T3 transfected with PADI4 expression vector containing a myc-His-tag® treated with 10 mM calcium chloride and 10 µM Ionomycin for 4 hours, whole cell lysate

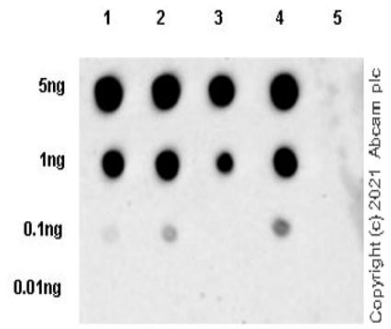
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 15 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

Exposure time: 48 seconds



Dot Blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

Dot blot analysis of Histone H3 (citrulline R2 + R8 + R17) using 281584 at 1/1000 (0.478 ug/ml) followed by a Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100,000 dilution.

Lane 1: Histone H3 (citrulline R2) peptide (aa 1-21)

Lane 2: Histone H3 (citrulline R8) peptide (aa 1-21)

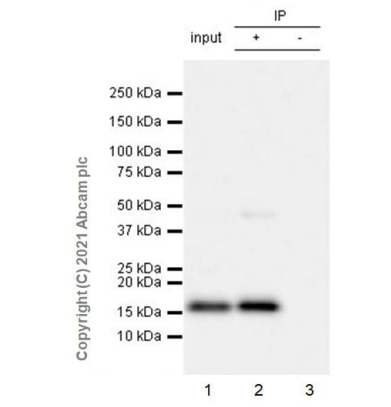
Lane 3: Histone H3 (citrulline R17) peptide (aa 1-21)

Lane 4: Histone H3 (citrulline R2/R8/R17) peptide (aa 1-21)

Lane 5: Unmodified histone H3 peptide (aa 1-21)

Exposure time: 3 minutes

Blocking and diluting buffer and concentration: 5% NFDM/TBST



Immunoprecipitation - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

Histone H3 (citrulline R2 + R8 + R17) was immunoprecipitated from 0.35 mg HEK-293T (human embryonic kidney) transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM Ionomycin for 4 hours, whole cell lysate 10 µg with 281584 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using 281584 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

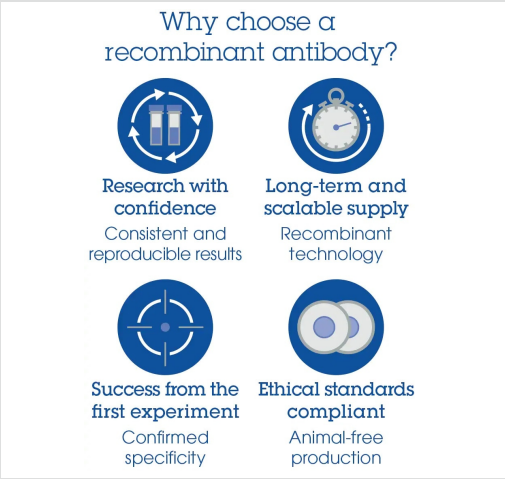
Lane 1: HEK-293T (human embryonic kidney) transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM Ionomycin for 4 hours, whole cell lysate 10 µg.

Lane 2: ab281584 IP in HEK-293T transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM Ionomycin for 4 hours whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab281584 in HEK-293T transfected with PADI4 expression vector containing a GFP-tag treated with 10 mM calcium chloride and 10 µM Ionomycin for 4 hours whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds



Anti-Histone H3 (citrulline R2 + R8 + R17) antibody [RM1001] (ab281584)

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