abcam

Product datasheet

Anti-Histone H3 (citrulline R2 + R8 + R17) antibody ab5103

27 Abreviews 487 References 7 Images

Overview

Product name	Anti-Histone H3 (citrulline R2 + R8 + R17) antibody
Description	Rabbit polyclonal to Histone H3 (citrulline R2 + R8 + R17)
Host species	Rabbit
Specificity	ab5103 detects a 17 kDa band in single lane Western Blot. Peptide inhibition in Western Blot hasn't been processed. Modification specificity is determined by Peptide Array. ab5103 binds strongly to Histone H3 citrulline 2 + 8 + 17 peptide. From Mar 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. You may also be interested in our alternative recombinant antibody, **ab281584**.
Tested applications	**Suitable for:** PepArr, IP, WB **Unsuitable for:** ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human, Recombinant fragment **Predicted to work with:** Rabbit, Cow, Monkey, a wide range of other species
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab32876**)
Positive control	WB: Mouse and rat brain tissue lysates. HL60 + DMSO, NIH/3T3 and PC12 whole cell lysates. ICC/IF: MCF7 cells. IP: HEK-293T (human embryonic kidney) transfected with PADI4 expression vector containing a GFP-tag
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 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.
Purity	Immunogen affinity purified
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab5103 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
PepArr		Use a concentration of 0.2 - 0.02 µg/ml.
IP		Use at an assay dependent concentration.
WB	★★★★☆ **(9)**	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa). **In our hands, when tested in western blot, this product typically gives a weaker signal in mouse and rat tissue lysates compared to mouse and rat cell lines. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented**

Application notes Is unsuitable for ICC/IF.

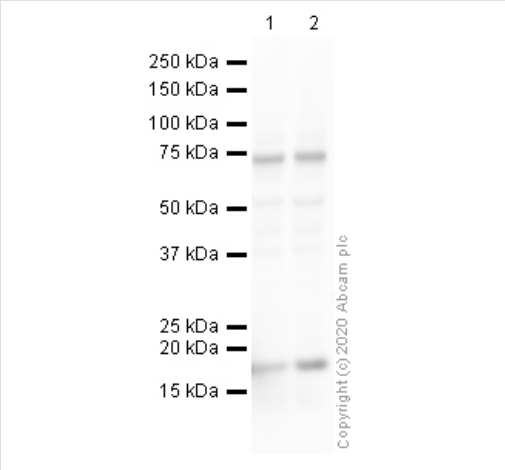
Target

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 (citrulline R2 + R8 + R17) antibody (ab5103)

All lanes : Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103) at 1 µg/ml

Lane 1 : Mouse brain tissue lysate
Lane 2 : Rat brain tissue lysate

Lysates/proteins at 10 µg per lane.

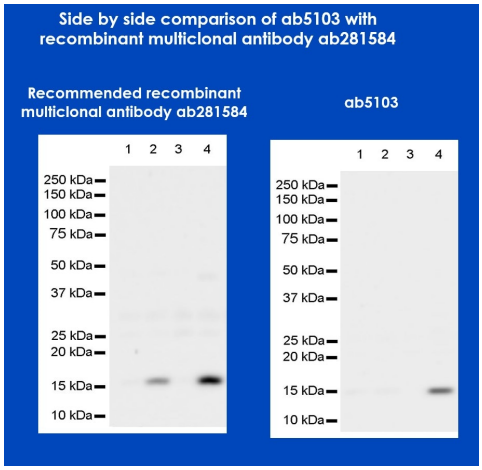
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

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

Exposure time: 1 minute

Blocking buffer: 2% BSA

Gel type: MES



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

This western blot image is a comparison between ab5103 and the alternative recombinant multiclonal antibody ab281584.

Left side - Recombinant multiclonal to Histone H3 (citrulline R2 + R8 + R17) - **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

Right side: Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

All lanes: Anti-Histone H3 (citrulline R2 + R8 + R17) antibody ab5103 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)

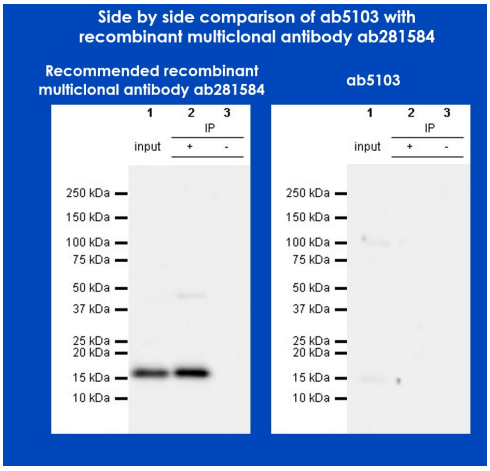
Why choose a recombinant antibody?

Research with confidence – consistent and reproducible results with every batch

Long-term and scalable supply – powered by recombinant technology for fast production

Success from the first experiment – confirmed specificity through extensive validation

Ethical standards compliant – production is animal-free



Immunoprecipitation - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

This immunoprecipitation image is a comparison between ab5103 and the alternative recombinant multiclonal antibody ab281584.

Left side - Recombinant multiclonal to Histone H3 (citrulline R2 + R8 + R17) - **ab281584**

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

Right side: Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

All lanes: Anti-Histone H3 (citrulline R2 + R8 + R17) antibody ab5103 at 1/1000 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: ab5103 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 ab5103 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

Secondary

All lanes: VeriBlot for IP Detection Reagent (HRP)(**ab131366**) was used at 1/5000 dilution.

Predicted band size: 15kDa

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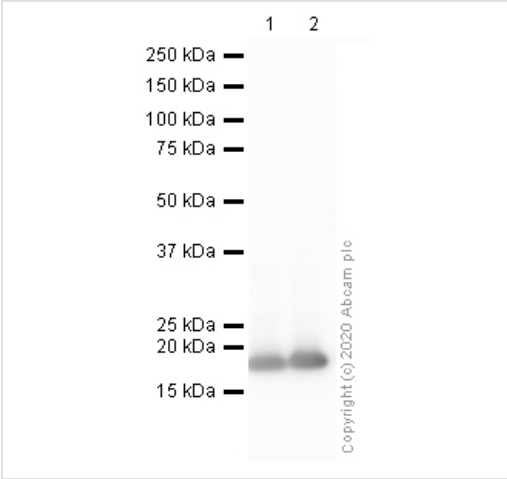
Research with confidence – consistent and reproducible results with every batch

Long-term and scalable supply – powered by recombinant technology for fast production

Success from the first experiment – confirmed specificity through extensive validation
Ethical standards compliant – production is animal-free



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

All lanes : Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103) at 1 µg/ml

Lane 1 : NIH/3T3 (Mouse embryo fibroblast cell line) nuclear lysate
Lane 2 : PC12 (Rat adrenal pheochromocytoma cell line) nuclear lysate

Lysates/proteins at 10 µg per lane.

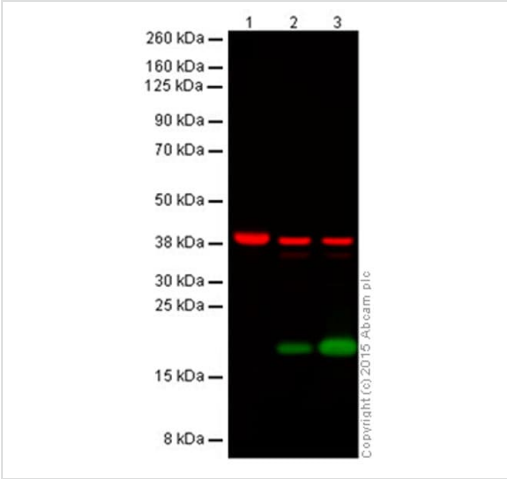
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

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

Exposure time: 1 minute

Blocking buffer: 2% BSA

Gel type: MES



Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

All lanes : Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103) at 0.2 µg/ml

Lane 1 : HL60 whole cell lysate (negative control)
Lane 2 : HL60 whole cell lysate + DMSO (solvent control)
Lane 3 : HL60 whole cell lysate + DMSO + Calcium Ionophore (positive control)

Lysates/proteins at 20 µg per lane.

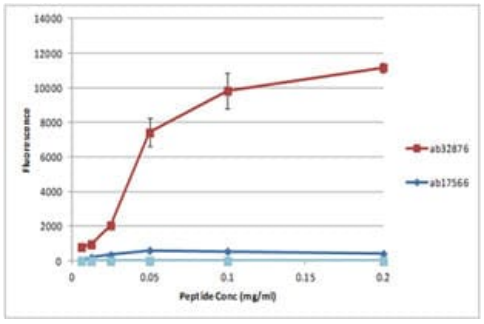
Secondary
All lanes : Goat anti Rabbit IR680 at 1/10000 dilution

Performed under reducing conditions.

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

Loading Control: GAPDH

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 Licor blocking buffer before being incubated with ab5103 overnight at 4°C. Antibody binding was detected using Goat anti Rabbit IR680 secondary at a 1:10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.



Peptide Array - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

All batches of ab5103 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 - citrulline 2 + 8 + 17 peptide (**ab32876**), indicating that this antibody specifically recognises the Histone H3 - citrulline 2 + 8 + 17 modifications.

ab32876 - Histone H3 - citrulline 2 + 8 + 17

ab17566 - Histone H3 - unmodified

Western blot - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103)

All lanes : Anti-Histone H3 (citrulline R2 + R8 + R17) antibody (ab5103) at 1 µg/ml

Lane 1 : HL60 (Human Caucasian promyelocytic leukaemia) DMSO and Calcium Ionophore treated Whole Cell Lysate with with 5% BSA
Lane 2 : HL60 (Human Caucasian promyelocytic leukaemia) DMSO and Calcium Ionophore treated Whole Cell Lysate with with 5% milk
Lane 3 : HL60 (Human Caucasian promyelocytic leukaemia) DMSO and Calcium Ionophore treated Whole Cell Lysate with with 3% milk

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 30 seconds

Abcam recommends using milk as the blocking agent. Abcam welcomes customer feedback and would appreciate any comments regarding this product and the data presented above .

Blots were developled with **Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody**

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