### Overview

#### Product name
Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade

#### Description
Rabbit polyclonal to Histone H3 (citrulline R2 + R8 + R17) - ChIP Grade

#### 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.

#### Tested applications
**Suitable for:** ICC/IF, PepArr, IHC-Fr, Flow Cyt, ChIP/Chip, WB, ChIP

#### Species reactivity
Reacts with: Mouse, Rat, Rabbit, Cow, Human, Monkey

**Predicted to work with:** a wide range of other species

#### Immunogen
Synthetic peptide corresponding to Human Histone H3 aa 1-100 (citrulline R2 + R8 + R17) conjugated to Keyhole Limpet Haemocyanin (KLH). Also SwissProt: P84243, Q71DB, Q16695, Q6NXT2.

Database link: P68431
(Peptide available as ab32876)

#### Positive control
This antibody gave a positive signal in HL60 Whole Cell Lysate - DMSO and Calcium Ionophore treated. In WB ab5103 only recognizes human or bovine histone H3 when PADI4 and calcium are added.

#### General notes
Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).

See other anti-rabbit secondary antibodies that can be used with this antibody.

### 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**

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.

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<th>Application</th>
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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. PubMed: 20733033</td>
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<tr>
<td>PepArr</td>
<td></td>
<td>Use a concentration of 0.2 - 0.02 µg/ml.</td>
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| IHC-Fr      | ⭐⭐⭐⭐⭐    | Use at an assay dependent concentration.  
*ab171870* - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody. |
| Flow Cyt    |           | Use at an assay dependent concentration. |
| ChIP/Chip   |           | Use at an assay dependent concentration. |
| WB          | ⭐⭐⭐⭐⭐    | Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).  
Abcam recommends using 3-5% milk as the blocking agent  
We recommend **Goat Anti-Rabbit IgG H&L (HRP) (ab97051)** secondary antibody. |
| ChIP        |           | Use at an assay dependent concentration. |

**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).  
Citrrullination 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 (H3K4me3), 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.

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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. Phosphorylation 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 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. 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
Chromatin immunoprecipitation using ab5103 on the pS2 promoter. Times are after stimulation by estrogen (Ul).

**All lanes**: Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (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

*why is the actual band size different from the predicted?*

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.
Immunocytochemistry/Immunofluorescence - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103)

This image is courtesy of an Anonymous Abreview.

ab5103 staining Histone H3 (citrulline 2 + 8 + 17) in Mouse bone marrow cells by Immunocytochemistry/Immunofluorescence. Cells were fixed in formaldehyde and permeabilized in 0.1% Triton X-100 prior to blocking in 5% Goat serum for 2 hours at 25°C. The primary antibody was diluted 1/250 in PBS and incubated with the sample for 12 hours at 4°C. The secondary antibody was Alexa Fluor® 488-conjugated Goat anti-Rabbit polyclonal, diluted 1/500. Nuclei were counterstained blue with DAPI.

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

Rabbit polyclonal to Histone H3 (citrulline 2 + 8 + 17) used at 1/2000 dilution, after blocking with TBST 5% BSA. Purified histones run out with approximately 250 ng of each histone.

Lanes 1-3 contain Histone H3 (250 ng per lane)
Lane 1: PADI4 + Calcium
Lane 2: H3 + PADI4
Lane 3: H3 + PADI4 + Calcium

Lanes 4-5 contain bulk histones (250 ng per lane)
Lane 4: PADI4
Lane 5: PADI4 + Calcium

Lane 6: MCF7 cell extract
Lane 7: MCF7 cell extract (HA-PADI4)

Secondary antibody: anti-rabbit HRP from Sigma.
In WB ab5103 only recognizes human or bovine histone H3 when PADI4 and calcium are added.

**Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (ab5103)**

Image of ab5103 stained human HeLa cells. The cells were PFA fixed (10 min), permabilised in TBS-T (20 min) and incubated with the antibody (ab5103, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to quench autofluorescence and block non-specific protein-protein interactions. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red). DAPI was used to stain the cell nuclei (blue).

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

**All lanes**: Anti-Histone H3 (citrulline R2 + R8 + R17) antibody - ChIP Grade (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 why is the actual band size different from the predicted?
**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 developed with Goat Anti-Rabbit IgG H&L (HRP) (ab97051) secondary antibody.

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**Please note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE"

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