Overview

Product name: Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] ab219407

Description: Rabbit monoclonal [EPR20358-120] to Histone H3 (citrulline R17)

Host species: Rabbit

Tested applications: Suitable for: ELISA, Flow Cyt (Intra), WB, IP, ICC/IF

Species reactivity: Reacts with: Mouse, Rat, Human

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

General notes: This 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 buffer: pH: 7.2
Preservative: 0.01% Sodium azide
Constituents: PBS, 40% Glycerol, 0.05% BSA

Purity: Protein A purified

Clone number: EPR20358-120

Isotype: IgG
The Abpromise guarantee

Our Abpromise guarantee covers the use of ab219407 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>
<th>Abreviews</th>
<th>Notes</th>
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<td>ELISA</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>Flow Cyt (Intra)</td>
<td>1/500. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.</td>
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<tr>
<td>WB</td>
<td>1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).</td>
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<tr>
<td>IP</td>
<td>1/30.</td>
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<tr>
<td>ICC/IF</td>
<td>1/100.</td>
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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. Phosphorylation at Ser-11 (H3S10ph) by MLTK isoform 1, RPS6KA5 or AURKB during mitosis or upon ultraviolet B irradiation. Phosphorylation at Thr-7 (H3T6ph) by PRKCB2 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**

ELISA analysis of Human Histone H3 (citrulline R17) recombinant protein at 100 ng/ml with ab219407. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as the secondary antibody.
**Western blot - Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (ab219407)**

**All lanes**: Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (ab219407) at 1/5000 dilution

**Lane 1**: HEK-293T (Human epithelial cell line from embryonic kidney) transfected with empty vector with GFP tag (vector control), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) for 2 hours, whole cell lysate

**Lane 2**: HEK-293T (Human epithelial cell line from embryonic kidney) transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (ab120116) 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 1/100000 dilution

**Predicted band size**: 15 kDa

**Observed band size**: 15 kDa

**Exposure time**: 3 seconds

**Blocking/Dilution buffer**: 5% BSA/TBST.

Histone H3R17 is citrullinated by PADI4 and CaCl2 is used as a cofactor according to the literature (PMID: 16567635). Ionomycin is used to improve the modification by PADI4 according to the literature (PMID: 26360112).
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (ab219407)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (Human epithelial cell line from embryonic kidney) cells transfected with GFP only or a GFP-tagged PADI4 expression construct, then treated with 10mM CaCl$_2$ for 2 hours, labeling Histone H3 (citrulline R17) with ab219407 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green).

Confocal image showing positive staining on HEK-293T cells transfected with a GFP-tagged PADI4 expression construct, then treated with 10mM CaCl$_2$ for 2 hours.

The nuclear counterstain is DAPI (blue).

Tubulin is detected with ab195889 (Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594)) at 1/200 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit IgG (Alexa Fluor® 488) (ab150077) at 1/1000 dilution.

Flow Cytometry (Intracellular) - Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (ab219407)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HEK-293T (Human epithelial cell line from embryonic kidney) transfected with empty vector (left panel) or PADI4 (WT, right panel), then treated with 10mM CaCl$_2$ and 10µM Ionomycin (ab120116) for 2 hours, labeling Histone H3 (citrulline R17) with ab219407 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 647) at 1/2000 dilution.

Positive signal is obtained from HEK-293T cells transfected with WT PADI4 treated with 10mM CaCl$_2$ and 10µM Ionomycin (ab120116) for 2 hours.
Histone H3 (citrulline R17) was immunoprecipitated from 0.35 mg of HEK-293T (Human epithelial cell line from embryonic kidney) transfected with PADI4 (WT), then treated with 10mM CaCl₂ and 10μM lonomycin (ab120116) for 2 hours, whole cell lysate with ab219407 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab219407 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

**Lane 1**: HEK-293T transfected with PADI4 (WT), then treated with 10mM CaCl₂ and 10μM lonomycin (ab120116) for 2 hours, whole cell lysate 10 µg (Input).

**Lane 2**: ab219407 IP in HEK-293T transfected with PADI4 (WT), then treated with 10mM CaCl₂ and 10μM lonomycin (ab120116) for 2 hours, whole cell lysate.

**Lane 3**: Rabbit monoclonal IgG (ab172730) instead of ab219407 in HEK-293T transfected with PADI4 (WT), then treated with 10mM CaCl₂ and 10μM lonomycin (ab120116) for 2 hours, whole cell lysate.

**Blocking/Dilution buffer**: 5% NFDM/TBST.

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All lanes: Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (ab219407) at 1/5000 dilution

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

**Lane 2**: NIH/3T3 (Mouse embryonic fibroblast cell line) transfected with PADI4 (WT) then treated with 10mM CaCl₂ for 2 hours, whole cell lysate

**Lane 3**: C6 (Rat glial tumor cell line) transfected with empty vector with GFP tag (vector control) then treated with 10mM CaCl₂ and 10μM lonomycin (ab120116) for 2 hours, whole cell lysate

**Lane 4**: C6 (Rat glial tumor cell line) transfected with PADI4 (WT), then treated with 10mM CaCl₂ and 10μM lonomycin (ab120116) 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
Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 1 second

Blocking/Dilution buffer: 5% BSA/TBST.

Lane 1: Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (ab219407) at 1/1000 dilution
Lane 2: Anti-Histone H3 (citrulline R17) antibody [EPR20358-120] (ab219407) at 1/200 dilution

Lane 1: E12 mouse embryo lysate
Lane 2: E12 rat embryo lysate

Lysates/proteins at 10 µg per lane.

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

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

Blocking/Dilution buffer: 5% BSA/TBST.

Exposure time: Lane 1: 1 second; Lane 2: 3 minutes.
Direct ELISA using ab219407 at 0-1000 ng/ml, followed by Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG(H+L) at 1/2500 dilution.

**Antigen concentration:** 1000 ng/ml.

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