abcam

Product datasheet

Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] ab219406

重组 RabMAb

1 References 9 图像

概述

产品名称	Anti-Histone H3 (citrulline R8)**抗体**[EPR20358-13]
描述	**兔单克隆抗体**[EPR20358-13] to Histone H3 (citrulline R8)
宿主	Rabbit
经测试应用	**适用于:** ELISA, Flow Cyt (Intra), IP, ICC/IF, WB
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR20358-13
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab219406**于以下的**经测试应用

"应用说明"**部分 下显示的**仅为**推荐的起始稀释度**;实际**最佳的稀释度/浓度应由使用者检定。**

应用	Ab评论	说明
ELISA		Use at an assay dependent concentration.

应用	Ab评论	说明
Flow Cyt (Intra)		1/500. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		1/30.
ICC/IF		1/100.
WB		1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).

靶标

功能

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.

序列相似性

Belongs to the histone H3 family.

发展阶段

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

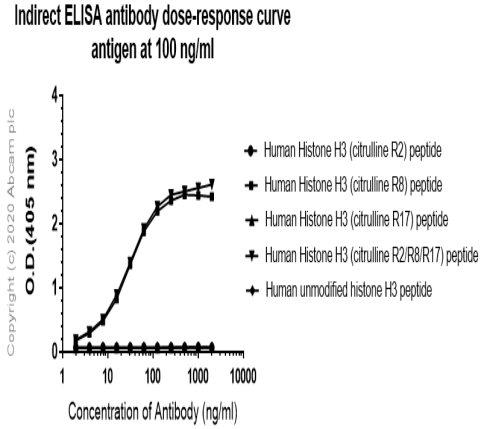
翻译后修饰

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.

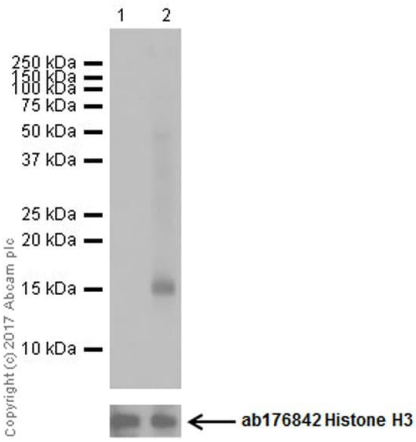
细胞定位 Nucleus. Chromosome.

图片



ELISA - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

ELISA analysis of Human Histone H3 (citrulline R8) recombinant protein at 100 ng/ml with ab219406. 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 R8) antibody [EPR20358-13] (ab219406)

All lanes : Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406) at 1/10000 dilution

Lane 1 : Whole cell lysate from 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
Lane 2 : Whole cell lysate from HEK-293T cells transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (**ab120116**) for 2 hours

Lysates/proteins at 10 µg per lane.

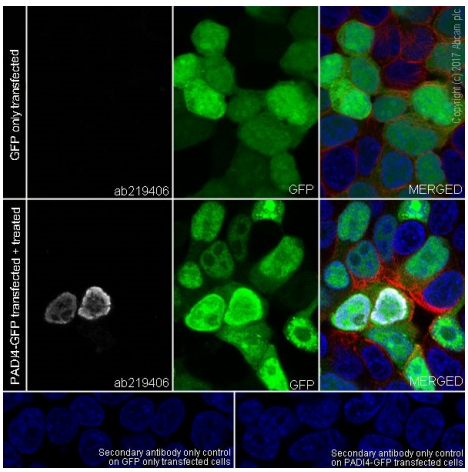
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 1 second

Blocking/Dilution buffer: 5% BSA/TBST.

Histone H3R8 is citrullinated by PADI4 and $CaCl_2$ 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 R8) antibody [EPR20358-13] (ab219406)

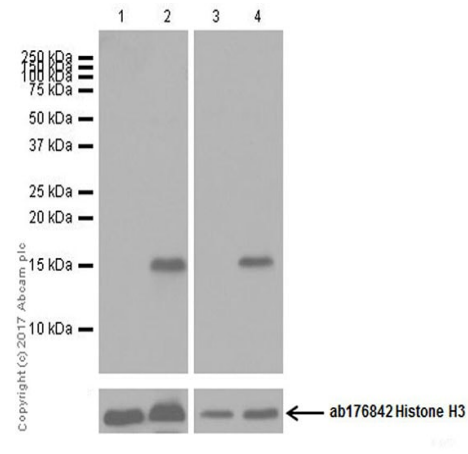
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HEK-293T (Human epithelial cell line from embryonic kidney) cells transfected with GFP only or a GFP-tagged PADI4 expression construct, then treated with 10mM $CaCl_2$ for 2 hours, labeling Histone H3 (citrulline R8) with ab219406 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.



Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

All lanes : Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406) at 1/5000 dilution

Lane 1 : Whole cell lysate from NIH/3T3 (Mouse embryonic fibroblast cell line) transfected with empty vector with GFP tag (vector control), then treated with 10mM CaCl2 for 2 hours
Lane 2 : Whole cell lysate from NIH/3T3 transfected with PADI4 (WT) then treated with 10mM CaCl2 for 2 hours
Lane 3 : Whole cell lysate from C6 (Rat glial tumor cell line) transfected with empty vector with GFP tag (vector control) then treated with 10mM CaCl2 and 10µM Ionomycin (**ab120116**) for 2 hours
Lane 4 : C6 transfected with PADI4 (WT), then treated with 10mM CaCl2 and 10µM Ionomycin (**ab120116**) for 2 hours

Lysates/proteins at 10 µg per lane.

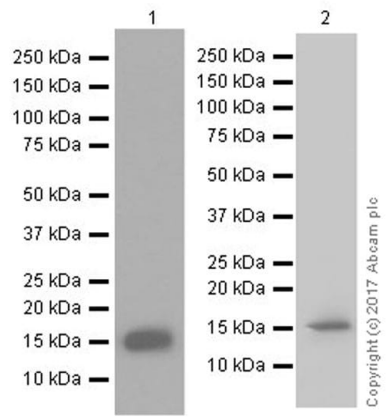
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at

1/100000 dilution

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

Exposure time: 1 second

Blocking/Dilution buffer: 5% BSA/TBST.



Western blot - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

All lanes : Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406) at 1/1000 dilution

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

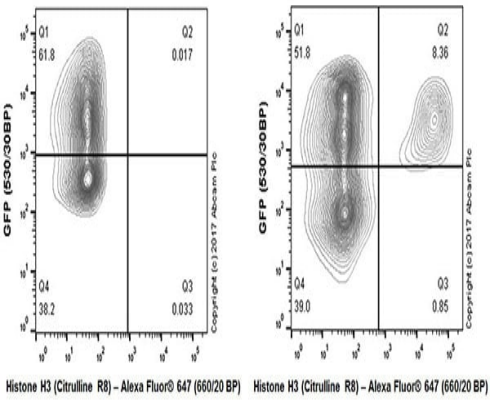
Lysates/proteins at 10 µg per lane.

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

Exposure time: 1 second

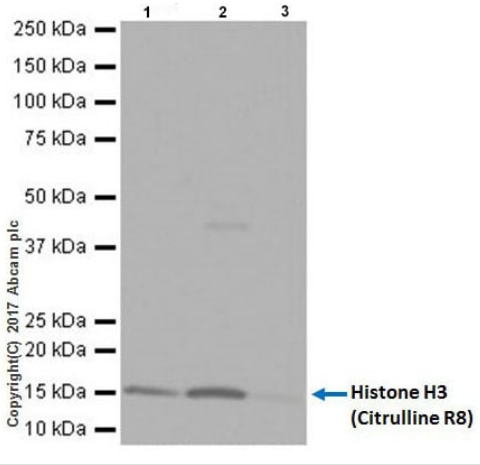
Blocking/Dilution buffer: 5% BSA/TBST.



Flow Cytometry (Intracellular) - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

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 CaCl2 and 10µM Ionomycin (**ab120116**) for 2 hours, labeling Histone H3 (citrulline R8)with ab219406 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 CaCl2 and 10µM Ionomycin (**ab120116**) for 2 hours.



Immunoprecipitation - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

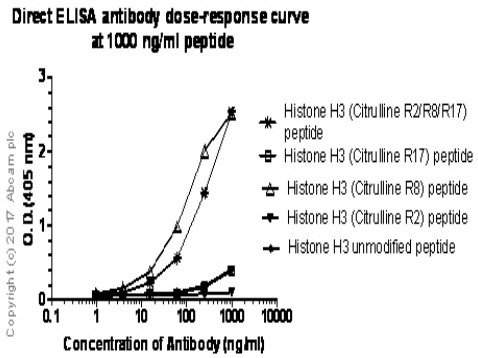
Histone H3 (citrulline R8) was immunoprecipitated from 0.35 mg of HEK-293T (Human epithelial cell line from embryonic kidney) transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (**ab120116**) for 2 hours, whole cell lysate with ab219406 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab219406 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 $CaCl_2$ and 10µM Ionomycin (**ab120116**) for 2 hours, whole cell lysate 10 µg (Input).
Lane 2: ab219406 IP in HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (**ab120116**) for 2 hours, whole cell lysate.
Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab219406 in HEK-293T transfected with PADI4 (WT), then treated with 10mM $CaCl_2$ and 10µM Ionomycin (**ab120116**) for 2 hours, whole cell lysate.

Blocking and dilution buffer: 5% NFDM/TBST.



ELISA - Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

Direct ELISA using ab219406 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.

Anti-Histone H3 (citrulline R8) antibody [EPR20358-13] (ab219406)

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