abcam

Product datasheet

Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)] ab177294

Recombinant RabMAb

6 Images

Overview

Product name	Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)]
Description	Rabbit monoclonal [EPR17684(2)] to Histone H3 (di methyl K56)
Host species	Rabbit
Tested applications	**Suitable for:** PepArr, WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human, Recombinant fragment
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa and NIH/3T3 whole cell lysates. ICC/IF: HeLa and NIH/3T3 cells.
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 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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17684(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab177294 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 at an assay dependent concentration.
WB		1/200. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ICC/IF		1/150.

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.

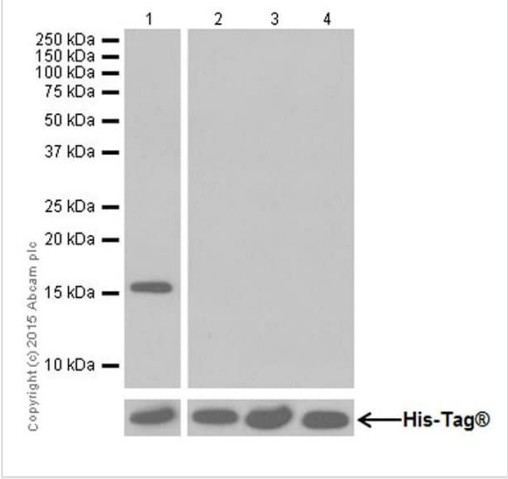
Developmental stage Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.

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 (di methyl K56) antibody [EPR17684(2)] (ab177294)

Lane 1 : Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)] (ab177294) at 1/5000 dilution
Lanes 2-4 : Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)] (ab177294) at 1/1000 dilution

Lane 1 : Recombinant mutant H3 chemically modified to mimic H3K56Me2
Lane 2 : Unmodified recombinant mutant H3
Lane 3 : Recombinant mutant H3 chemically modified to mimic H3K56Me1
Lane 4 : Recombinant mutant H3 chemically modified to mimic H3K56Me3

Lysates/proteins at 0.01 µg per lane.

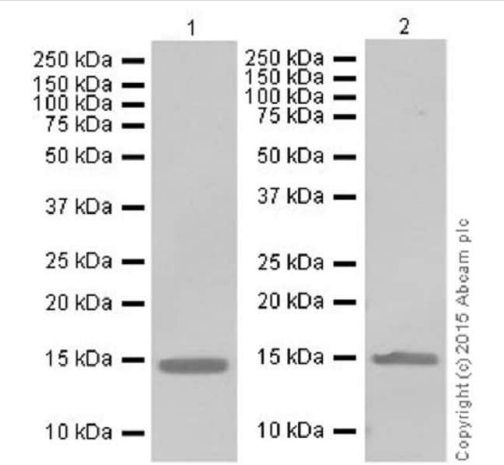
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 3 minutes

Blocking buffer 5% BSA/TBST

Diluting buffer 5% BSA/TBST



Western blot - Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)] (ab177294)

All lanes : Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)] (ab177294) at 1/200 dilution

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell lysate

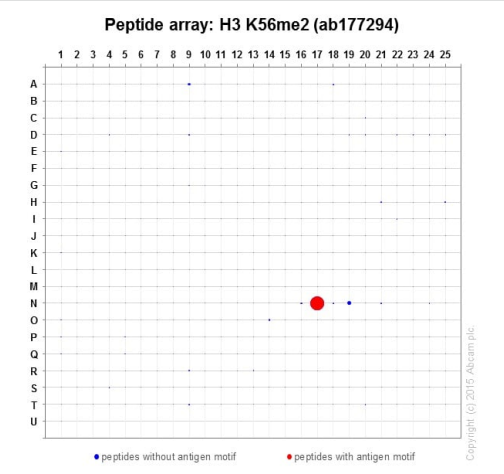
Lysates/proteins at 10 µg per lane.

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 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

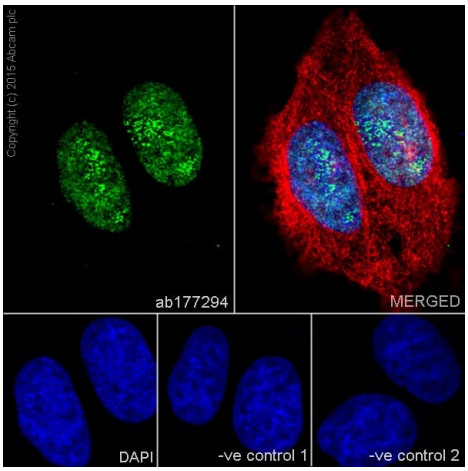


Peptide Array - Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)] (ab177294)

ab177294 was 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**.

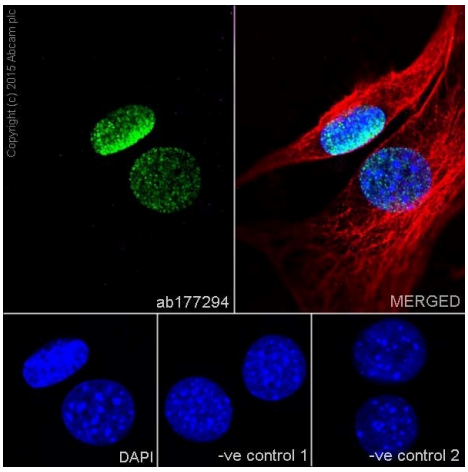


Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)] (ab177294)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Histone H3 (di methyl K56) with ab177294 at 1/150 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:
-ve control 1: ab177294 at 1/150 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) at 1/1000 dilution.
-ve control 2: Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) at 1/1000 dilution.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (di methyl K56) antibody [EPR17684(2)] (ab177294)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling Histone H3 (di methyl K56) with ab177294 at 1/150 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on NIH/3T3 cell line. The nuclear counter stain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb (**ab7291**) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (**ab150120**) at 1/1000 dilution (red).

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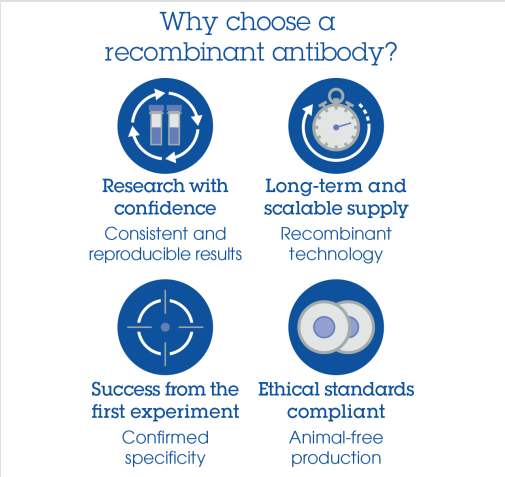

Anti-Histone H3 (di methyl K56) antibody

[EPR17684(2)] (ab177294)

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