abcam

Product datasheet

Anti-Histone H3 (asymmetric di methyl R17) antibody ab8284

17 Abreviews 57 References 6 Images

Overview

Product name	Anti-Histone H3 (asymmetric di methyl R17) antibody
Description	Rabbit polyclonal to Histone H3 (asymmetric di methyl R17)
Host species	Rabbit
Specificity	Peptide competition experiments confirmed that the antibody recognises specifically methylated R17 in H3 and not unmethylated H3 or methylated R3 in H4 (see figure 1). In whole cell extract the antibody recognises specifically only the methylated histone H3 protein band (see figure 2) Further, the antibody doesn't crossreact with the C-terminal methylation sites of CARM1 in histone H3. In IHC on paraffin-embedded sections of human tonsil, the antibody shows nuclear staining across most nuclei. Slight batch to batch variation is observed, but no more than 50% cross reactivity with symmetric di methyl R17 peptide is allowed.
Tested applications	**Suitable for:** PepArr, IHC-P, ICC/IF, Dot blot, WB
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat, Chicken, Cow, Caenorhabditis elegans, Drosophila melanogaster, Mammals, Toxoplasma gondii
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	The nuclear hormone receptor co-activator CARM1 has the potential to methylate histone H3 at arginine residues in vitro. The methyltransferase activity of CARM1 is necessary for its co-activator functions in transient transfection assays. However, the role of this methyltransferase in vivo is unclear, given that methylation of arginines is not easily detectable on purified histones. This antibody recognizes methylated arginine 17 (R17) of histone H3, the major site of methylation by CARM1. Bauer et al (2001) have shown by using this antibody that methylated R17 exists in vivo. Chromatin immunoprecipitation analysis shows that R17 methylation on histone H3 is dramatically upregulated when the estrogen receptor-regulated pS2 gene is stimulated by estradiol and TPA. Coincident with the appearance of methylated R17, the CARM1 methyltransferase is found associated with the histones on the pS2 gene. Together these results demonstrate that the CARM1 methyltransferase is recruited to an active promoter and that CARM1-mediated methylation of histone H3 at R17 takes place in vivo during this active state. 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
Primary antibody notes	The nuclear hormone receptor co-activator CARM1 has the potential to methylate histone H3 at arginine residues in vitro. The methyltransferase activity of CARM1 is necessary for its co-activator functions in transient transfection assays. However, the role of this methyltransferase in vivo is unclear, given that methylation of arginines is not easily detectable on purified histones. This antibody recognizes methylated arginine 17 (R17) of histone H3, the major site of methylation by CARM1. Bauer et al (2001) have shown by using this antibody that methylated R17 exists in vivo. Chromatin immunoprecipitation analysis shows that R17 methylation on histone H3 is dramatically upregulated when the estrogen receptor-regulated pS2 gene is stimulated by estradiol and TPA. Coincident with the appearance of methylated R17, the CARM1 methyltransferase is found associated with the histones on the pS2 gene. Together these results demonstrate that the CARM1 methyltransferase is recruited to an active promoter and that CARM1-mediated methylation of histone H3 at R17 takes place in vivo during this active state.
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab8284 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 0.002 - 0.0002 µg/ml.
IHC-P	★★★★★ (2)	Use at an assay dependent concentration.
ICC/IF	★★★★☆ (3)	Use a concentration of 0.1 µg/ml.
Dot blot		Use at an assay dependent concentration.
WB	★★★★☆ (9)	1/1000 - 1/2000.

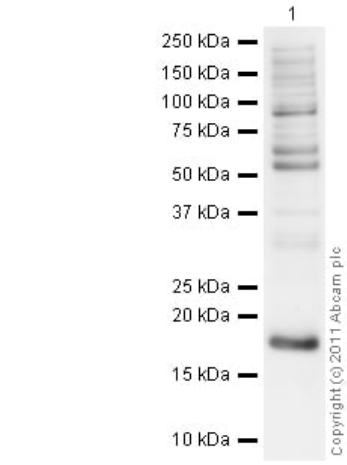
Target

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 (asymmetric di methyl R17) antibody (ab8284)

Anti-Histone H3 (asymmetric di methyl R17) antibody (ab8284) at 1 µg/ml + HeLa Histone Preparation Nuclear Lysate at 2.5 µg/ml

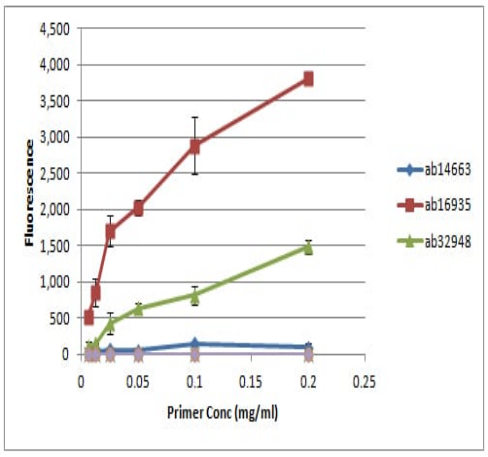
Secondary
Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (**ab97080**) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15.4 kDa
Observed band size: 17 kDa
Additional bands at: 55 kDa, 60 kDa, 90 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes



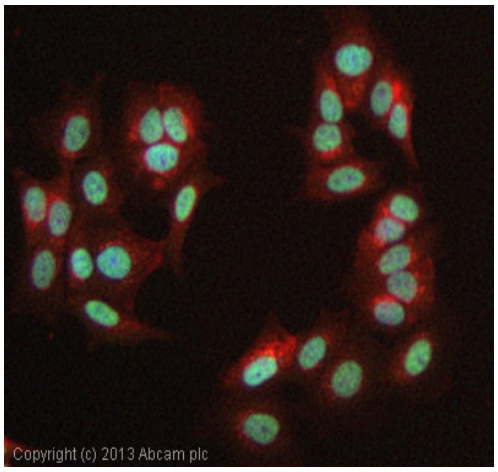
Peptide Array - Anti-Histone H3 (asymmetric di methyl R17) antibody (ab8284)

All batches of ab8284 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 - asymmetric di methyl R17 peptide (**ab16935**), indicating that this antibody specifically recognises the Histone H3 - asymmetric di methyl R17 modification.

ab16935 - Histone H3 - asymmetric di methyl R17

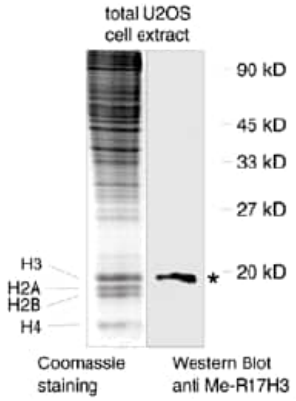
ab32948 - Histone H3 - symmetric di methyl R17

ab14663 - Histone H3 - unmodified



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (asymmetric di methyl R17) antibody (ab8284)

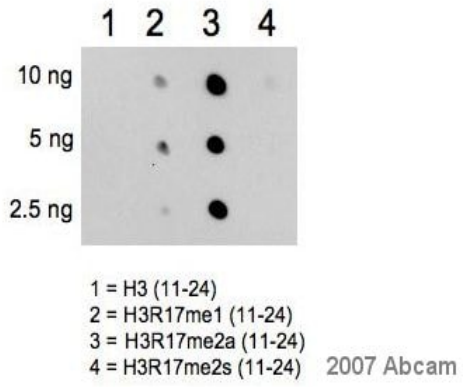
ICC/IF image of ab8284 stained MCF7 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab8284, 0.1µg/ml) overnight at +4°C. The secondary antibody (green) was **ab96899**, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-Histone H3 (asymmetric di methyl R17) antibody (ab8284)

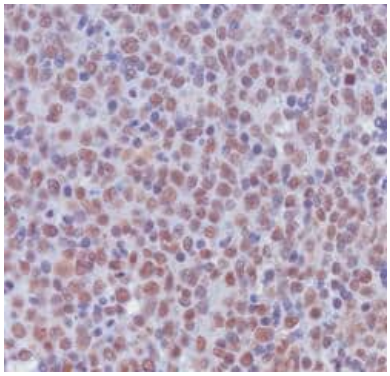
Taken from Bauer et al, (2001).

Total U2OS cell extract was western blotted using the anti-Me-R17H3 antibody. The asterisk indicates methylated histone H3. The left panel shows presence of core histones (indicated on the left) by Coomassie Blue staining. Molecular weights are indicated on the right.



Dot Blot - Anti-Histone H3 (asymmetric di methyl R17) antibody (ab8284)

A dot blot was performed using unmodified peptide (lane 1), Histone H3 mono methyl R17 peptide (lane 2), Histone H3 asymmetric di methyl R17 peptide (lane 3) and Histone H3 symmetric di methyl R17 peptide (lane 4). The dot blot indicates that ab8284 is specific to Histone H3 asymmetric di methyl R17.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (asymmetric di methyl R17) antibody (ab8284)

ab8284 was used in immunohistochemistry with paraffin embedded sections of human tonsil, using DAB as a chromogen (brown). Counterstaining of nuclei was performed with haemotoxylin (blue).

Staining is seen confined to the nucleus.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors