# abcam

# Product datasheet

# Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] ab177284



# 10 Images

#### Overview

**Product name** Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719]

**Description** Rabbit monoclonal [EPR17719] to Histone H3 (symmetric di methyl R26)

**Host species** Rabbit

**Tested applications** Suitable for: WB, IHC-P, ICC/IF, PepArr

Species reactivity Reacts with: Mouse, Rat, Human

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

Positive control WB: Human ovary lysate; NTera-2/D1, HL-60, HeLa, F9, D3 and NIH/3T3 whole cell lysates. IHC-

P: Human stomach, rat colon and mouse colon tissues. 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, 40% Glycerol (glycerin, glycerine), 0.05% BSA

**Purity** Protein A purified

Clonality Monoclonal Clone number EPR17719

Isotype ΙgG

### **Applications**

# The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab177284 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
WB		1/2000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/1000.
PepArr		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

## **Developmental stage**

Belongs to the histone H3 family.

# Post-translational

modifications

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

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 PAD4 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.

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

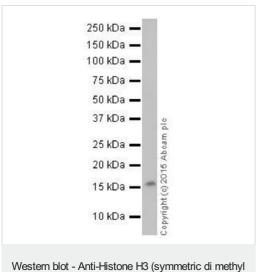
ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate

DNA accessibility to repair proteins.

#### Cellular localization

Nucleus. Chromosome.

### **Images**



Western blot - Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] (ab177284)

Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] (ab177284) at 1/2000 dilution + Human ovary lysate at 10 µg

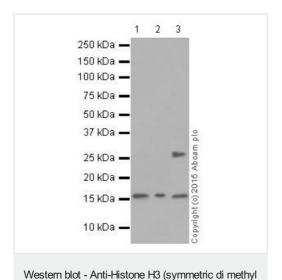
#### Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/50000 dilution

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

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% BSA/TBST.



R26) antibody [EPR17719] (ab177284)

**All lanes :** Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] (ab177284) at 1/2000 dilution

**Lane 1 :** NTera-2/D1 (Human malignant pluripotent embryonic carcinoma) whole cell lysate

**Lane 2**: HL-60 (Human promyelocytic leukemia cells) whole cell lysate

**Lane 3**: HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate

Lysates/proteins at 10 µg per lane.

# Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

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

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% BSA/TBST.

1 2 3
250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —
15 kDa —
10 kDa —

Western blot - Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] (ab177284)

**All lanes :** Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] (ab177284) at 1/2000 dilution

**Lane 1 :** F9 (Mouse embryonic carcinoma cell line) whole cell lysate

Lane 2: D3 (Mouse embryonic stem cell) whole cell lysate

Lane 3: NIH/3T3 (Mouse embryonic fibroblast cells) whole cell lysate

Lysates/proteins at 10 µg per lane.

# Secondary

**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

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

Exposure time: 10 seconds

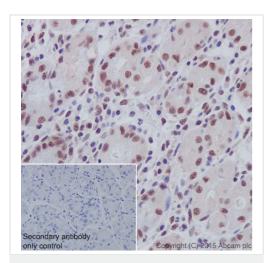
Blocking/Dilution buffer: 5% BSA/TBST.

Peptide Array - Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] (ab177284)

ab177284 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 <a href="here">here</a>.

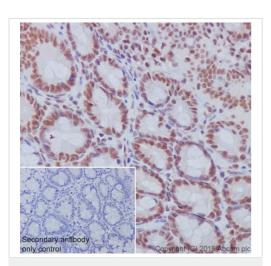


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (symmetric dimethyl R26) antibody [EPR17719] (ab177284)

Immunohistochemical analysis of paraffin-embedded human stomach tissue labeling Histone H3 (symmetric di methyl R26) with ab177284 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on human stomach tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

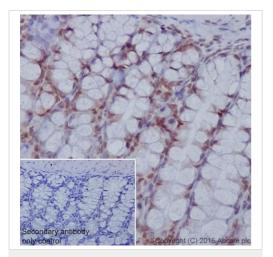


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (symmetric dimethyl R26) antibody [EPR17719] (ab177284)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling Histone H3 (symmetric di methyl R26) with ab177284 at 1/100 dilution, followed by Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on rat colon tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

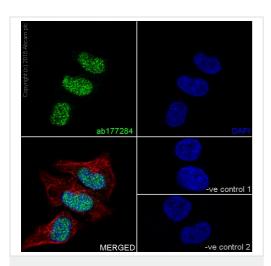


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 (symmetric dimethyl R26) antibody [EPR17719] (ab177284)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Histone H3 (symmetric di methyl R26) with ab177284 at 1/100 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Nuclear staining on mouse colon tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] (ab177284)

ab177284 DAPI

-ve control 1

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (symmetric di methyl R26) antibody [EPR17719] (ab177284)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H3 (symmetric di methyl R26) with ab177284 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on HeLa cell line. The nuclear counterstain 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<sup>®</sup> 594) (ab150120) secondary at 1/1000 dilution (red). The negative controls are as follows:-

-ve control 1: ab177284 at 1/1000 dilution followed by Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 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<sup>®</sup> 488) (ab150077) at 1/1000 dilution.

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labeling Histone H3 (symmetric di methyl R26) with ab177284 at 1/1000 dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing nuclear staining on NIH/3T3 cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin mouse MAb ( $\underline{ab7291}$ ) at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor  $^{\circledR}$  594) ( $\underline{ab150120}$ ) secondary antibody at 1/1000 dilution (red).

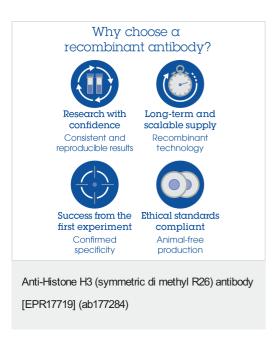
The negative controls are as follows:-

-ve control 1: ab177284 at 1/1000 dilution, followed by Goat Anti-Mouse IgG H&L (Alexa Fluor<sup>®</sup> 594) (ab150120) secondary antibody at 1/1000 dilution.

-ve control 2: Anti-alpha Tubulin mouse MAb (ab7291) at 1/1000

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dilution, followed by Goat Anti-Rabbit lgG H&L (Alexa Fluor<sup>®</sup> 488) (ab150077) secondary antibody at 1/1000 dilution.



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