abcam

Product datasheet

Anti-Histone H3.3 antibody ab94817

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Overview

Product name Anti-Histone H3.3 antibody

Description Rabbit polyclonal to Histone H3.3

Host species Rabbit

SpecificityThis antibody was raised against a peptide derived from within residues 50 to the C-terminus of

Human Histone H3.3. However, due to the extensive amount of homology between H3 variants,

we expect this antibody to show some cross reactivity with H3.1 and H3.2.

Tested applications Suitable for: WB

Species reactivity Reacts with: Mouse, Cow, Human

Predicted to work with: Rat, Rabbit, Chicken, Pig, Zebrafish, Orangutan

Immunogen Synthetic peptide corresponding to Human Histone H3.3 aa 50 to the C-terminus conjugated to

keyhole limpet haemocyanin. Database link: <u>P84243</u>

(Peptide available as ab129219)

Positive control This antibody gave a positive signal in the following whole cell lysates: HeLa; SAOS-2; Jurkat;

NIH3T3.

General notes

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

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab94817 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	★★☆☆☆(2)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).

Target

Function

Variant histone H3 which replaces conventional H3 in a wide range of nucleosomes in active genes. Constitutes the predominant form of histone H3 in non-dividing cells and is incorporated into chromatin independently of DNA synthesis. Deposited at sites of nucleosomal displacement throughout transcribed genes, suggesting that it represents an epigenetic imprint of transcriptionally active chromatin. 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 throughout the cell cycle independently of DNA synthesis.

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 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.

Specifically enriched in modifications associated with active chromatin such as methylation at Lys-5 (H3K4me), Lys-37 and Lys-80. 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), which are linked to gene repression, are underrepresented.

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.

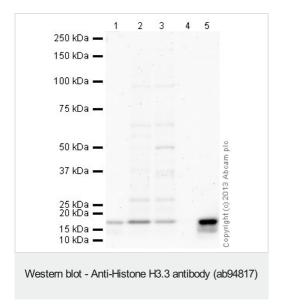
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and 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 Tvr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin. Phosphorylation on Ser-32 (H3S31ph) is specific to regions bordering centromeres in metaphase chromosomes.

Ubiquitinated. Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination.

Cellular localization

Nucleus. Chromosome.

Images



All lanes: Anti-Histone H3.3 antibody (ab94817) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5 μg

Lane 2: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell

Lysate at 20 µg

Lane 3: HeLa (Human epithelial carcinoma cell line) Whole Cell

Lysate at 20 µg

Lane 4: Histone H3.1 Recombinant Protein at 0.1 µg

Lane 5: Histone H3.3 Recombinant Protein at 0.1 µg

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) preadsorbed (**ab97080**) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa **Observed band size:** 17 kDa

Exposure time: 20 minutes

All lanes: Anti-Histone H3.3 antibody (ab94817) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2: HeLa Histone Preparation Nuclear Lysate

Lane 3 : Saos 2 (Human epithelial-like osteosarcoma cell line)
Whole Cell Lysate

Lane 4: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

Lane 5 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Histone H3.3 peptide ($\underline{ab129219}$) at 1 $\mu g/ml$

Lane 6 : HeLa Histone Preparation Nuclear Lysate with Histone H3.3 peptide (ab129219) at 1 μ g/ml

Lane 7: Saos 2 (Human epithelial-like osteosarcoma cell line)
Whole Cell Lysate with Histone H3.3 peptide (**ab129219**) at 1 μg/ml **Lane 8**: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell

Lysate with Histone H3.3 peptide (ab129219) at 1 µg/ml

Lane 9 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate with Histone H3.1 Peptide at 1 μ g/ml

Lane 10 : HeLa Histone Preparation Nuclear Lysate with Histone H3.1 Peptide at 1 μ g/ml

 $\textbf{Lane 11:} \ Saos\ 2\ (Human\ epithelial-like\ osteosarcoma\ cell\ line)$ Whole Cell Lysate with Histone H3.1 Peptide at 1 $\mu g/ml$

Lane 12: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate with Histone H3.1 Peptide at 1 µg/ml

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

L1 L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12

250—
150—
100—
75—
50—
37—

25—
20—
15—
10—

Western blot - Anti-Histone H3.3 antibody (ab94817)

Predicted band size: 15 kDa

Exposure time: 12 minutes

All lanes: Anti-Histone H3.3 antibody (ab94817) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2: Saos 2 (Human epithelial-like osteosarcoma cell line) Whole Cell Lysate

Lane 3 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lane 4: NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Western blot - Anti-Histone H3.3 antibody (ab94817)

L1 L2

250-150L3 L4

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa **Observed band size:** 17 kDa

Additional bands at: 100 kDa, 50 kDa, 70 kDa. We are unsure as

to the identity of these extra bands.

Exposure time: 8 minutes

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