Product datasheet

Anti-Histone H3 (di methyl K4) antibody - ChIP Grade
ab7766

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Overview

Product name
Anti-Histone H3 (di methyl K4) antibody - ChIP Grade

Description
Rabbit polyclonal to Histone H3 (di methyl K4) - ChIP Grade

Host species
Rabbit

Tested applications
Suitable for: ChIP, Dot blot, WB, ChIP/Chip, PepArr, IHC-Fr, ICC/IF, IHC-P

Species reactivity
Reacts with: Mouse, Human, Pig, Saccharomyces cerevisiae, Tetrahymena, Drosophila melanogaster, Schizosaccharomyces pombe, Plasmodium falciparum, Common marmoset, Candida albicans

Predicted to work with: Mammals

Immunogen
Synthetic peptide within Human Histone H3 aa 1-100 (di methyl K4) conjugated to Keyhole Limpet Haemocyanin (KLH). The exact sequence is proprietary. (Peptide available as ab7768, ab125304, ab7766)

Positive control
Calf Thymus Histone Preparation ICC-IF: Hela cells.

General notes
For detection of Histone H3 specifically methylated at position Lys 4. This antibody was used in a screen by Dover et al, (2002) to isolate yeast mutants that are unable to methylate Lysine 4. In immunofluorescence, this antibody detects foci in the nucleus that are non-colocalising with condensed chromatin. The perinuclear and perinucleolar heterochromatin are not stained with this antibody.

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

For detection of Histone H3 specifically methylated at position Lys 4. This antibody was used in a screen by Dover et al, (2002) to isolate yeast mutants that are unable to methylate Lysine 4. In immunofluorescence, this antibody detects foci in the nucleus that are non-colocalising with condensed chromatin. The perinuclear and perinucleolar heterochromatin are not stained with this antibody.

Clonality

Polyclonal

Isotype

IgG

Applications

Our Abpromise guarantee covers the use of ab7766 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
<thead>
<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ChIP</td>
<td></td>
<td>Use 2 µg for 25 µg of chromatin.</td>
</tr>
<tr>
<td>Dot blot</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>WB</td>
<td></td>
<td>Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa). Can be blocked with Human Histone H3 (di methyl K4) peptide (ab7768).</td>
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<tr>
<td>ChIP/Chip</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>PepArr</td>
<td></td>
<td>Use a concentration of 0.2 - 2 µg/ml.</td>
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<tr>
<td>IHC-Fr</td>
<td></td>
<td>1/1000.</td>
</tr>
<tr>
<td>ICC/IF</td>
<td></td>
<td>Use a concentration of 0.1 - 1 µg/ml.</td>
</tr>
<tr>
<td>IHC-P</td>
<td></td>
<td>1/800.</td>
</tr>
</tbody>
</table>

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 PRKCB2 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.
Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab7766 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.

All batches of ab7766 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 - di methyl K4 peptide (ab7768), indicating that this antibody specifically recognises the Histone H3 - di methyl K4 modification.

- ab1340 - Histone H3 - mono methyl K4
- ab1342 - Histone H3 - tri methyl K4
- ab1771 - Histone H3 - mono methyl K9
- ab1772 - Histone H3 - di methyl K9
- ab1773 - Histone H3 - tri methyl K9
- ab1780 - Histone H3 - mono methyl K27
- ab1781 - Histone H3 - di methyl K27
- ab1782 - Histone H3 - tri methyl K27
ab7228 - Histone H3 - unmodified

ab7768 - Histone H3 - di methyl K4

ab7766 stained in Hela cells. Cells were fixed with 100% methanol (5 min) at room temperature and incubated with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab7766 at 0.2 µg/ml and ab7291 (Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control) at 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150120 (pseudo-colored red) and ab150081 (colored green) used at 1 µg/ml for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 µM for 1 hour at room temperature.

Dimethylated lysine 4 (green) is found in several hundred small nuclear foci that do not colocalize with condensed regions of chromatin (DAPI stained, red). The perinuclear and perinucleolar heterochromatin do not stain with this antibody.
Western blot - Anti-Histone H3 (di methyl K4) antibody - ChIP Grade (ab7766) at 1 µg/ml + Calf Thymus Histone Preparation Nuclear Lysate (ab121) at 0.5 µg

**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 kDa
**Observed band size:** 17 kDa

*why is the actual band size different from the predicted?*

**Additional bands at:** 14 kDa. We are unsure as to the identity of these extra bands.

**Exposure time:** 4 minutes

ab7766 at 1/200 staining human U2OS (osteosarcoma) cells by ICC/IF. The cells were paraformaldehyde fixed and then stained with the antibody for 1 hour. A Cy2® conjugated donkey anti-rabbit antibody was used as the secondary (green). The image shows uniformal staining of the whole nucleus, with several species found. The insert shows H3-di methyl K4 of tw2 cells only. DAPI nuclear staining is shown in blue.
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H3 (di methyl K4) antibody - ChIP Grade (ab7766)

This image is courtesy of an anonymous abreview.

Western blot - Anti-Histone H3 (di methyl K4) antibody - ChIP Grade (ab7766)

MCF7 cells were incubated at 37\degree C for 24h with vehicle control (0 \&microM) and different concentrations of tranylcypromine hydrochloride (ab120606). Increased expression of Histone 3 K4 di-methyl (ab7766) in MCF7 cells correlates with an increase in tranylcypromine hydrochloride concentration, as described in literature.

Nuclear extracts were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 10\&microg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab7766 at 1 \&microg /ml and ab1791 at 1 \&microg /ml overnight at 4\degree C. Antibody binding was detected using an anti-rabbit HRP secondary antibody (ab97051) at 1/10000 dilution and visualised using ECL development solution.

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