Product datasheet

Anti-Histone H4 antibody - ChIP Grade ab7311

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Overview

Product name  Anti-Histone H4 antibody - ChIP Grade
Description  Rabbit polyclonal to Histone H4 - ChIP Grade
Host species  Rabbit
Specificity  Histone H4
Tested applications  Suitable for: IHC-P, ICC/IF, ChIP, WB
Species reactivity  Reacts with: Mouse, Cow, Human, Saccharomyces cerevisiae
Predicted to work with: Mammals  ▲
Immunogen  Synthetic peptide corresponding to Human Histone H4 aa 1-100 conjugated to keyhole limpet haemocyanin.
Database link: P62805
(Peptide available as ab21044)
Positive control  Calf Thymus Histone Preparation; Hela whole cell extract

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  Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4
Purity  Immunogen affinity purified
Clonality  Polyclonal
Isotype  IgG

Applications

Our Abpromise guarantee covers the use of ab7311 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
**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 H4 family.

**Post-translational modifications**
- Acetylation at Lys-6 (H4K5ac), Lys-9 (H4K8ac), Lys-13 (H4K12ac) and Lys-17 (H4K16ac) occurs in coding regions of the genome but not in heterochromatin.
- Citrullination at Arg-4 (H4R3ci) by PADI4 impairs methylation.
- Monomethylation and asymmetric dimethylation at Arg-4 (H4R3me1 and H4R3me2a, respectively) by PRMT1 favors acetylation at Lys-9 (H4K8ac) and Lys-13 (H4K12ac).
- Demethylation is performed by JMJD6. Symmetric dimethylation on Arg-4 (H4R3me2s) by the PRDM1/PRMT5 complex may play a crucial role in the germ-cell lineage.
- Monomethylated, dimethylated or trimethylated at Lys-21 (H4K20me1, H4K20me2, H4K20me3). Monomethylation is performed by SET8. Trimethylation is performed by SUV420H1 and SUV420H2 and induces gene silencing.
- 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. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage. The exact role of H4K91ub1 in DNA damage response is still unclear but it may function as a licensing signal for additional histone H4 post-translational modifications such as H4 Lys-21 methylation (H4K20me).
- Sumoylated, which is associated with transcriptional repression.

**Cellular localization**
Nucleus. Chromosome.

<table>
<thead>
<tr>
<th>Application</th>
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<th>Notes</th>
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<tr>
<td>IHC-P</td>
<td>Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
<td></td>
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<tr>
<td>ICC/IF</td>
<td>😄😄😄😄</td>
<td>1/200.</td>
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<tr>
<td>ChIP</td>
<td>😄😄😄😄</td>
<td>Use 2 µg for 25 µg of chromatin.</td>
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<td>WB</td>
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1/500 - 1/1000. Detects a band of approximately 14 kDa (predicted molecular weight: 11 kDa).

**Images**
Chromatin was prepared from Hela cells according to the Abcam X-ChiP protocol. Cells were fixed with formaldehyde for 10min. The ChiP was performed with 25µg of chromatin, 2µg of ab7311 (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). Primers and probes are located in the first kb of the transcribed region.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 antibody - ChiP Grade (ab7311)

IHC image of Histone H4 staining in human breast carcinoma FFPE section, performed on a Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab7311, 1µg/ml, for 8 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

All lanes : Anti-Histone H4 antibody - ChiP Grade (ab7311) at 1/500 dilution

Lane 1 : Calf thymus histone extract
Lane 2 : Calf thymus histone extract with Human Histone H4 peptide (ab21044) at 1 µg/ml
Lane 3 : Calf thymus histone extract with Histone H4 peptide - acetyl K16 (ab22376) at 1 µg/ml
Lane 4 : Calf thymus histone extract with Human Histone H4 (acetyl K20) peptide (ab20632) at 1 µg/ml

Lysates/proteins at 0.5 µg per lane.
Secondary

All lanes: Alexa-Fluor Goat anti-rabbit IgG at 1/10000 dilution

Predicted band size: 11 kDa
Observed band size: 14 kDa

Why is the actual band size different from the predicted?

SKN-SH cells were fixed in 4% paraformaldehyde for 10 mins, permeabilized in PBS-0.5% Triton X-100 for 5 mins and incubated for 30 minutes with ab7311 (1/200 dilution). The slides were rinsed once in PBS-Triton (0.1%), twice in PBS then incubated with the secondary antibody for 30 mins. The DNA is stained with DAPI (blue). Clear nuclear staining with ab7311 can be seen (green).

Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 antibody - ChIP Grade (ab7311)
This image is courtesy of Darin McDonald

Western blot - Anti-Histone H4 antibody - ChIP Grade (ab7311) at 1/500 dilution + Human Bone Whole Tissue Lysate at 25 µg with BSA, 1 Hour, 23°C at 5 %

Developed using the ECL technique.

Predicted band size: 11 kDa

Exposure time: 1 minute

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