**Product datasheet**

**Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade ab9051**

<table>
<thead>
<tr>
<th>Rating</th>
<th>Reviews</th>
<th>References</th>
<th>Images</th>
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<tbody>
<tr>
<td>★★★★★</td>
<td>15</td>
<td>76</td>
<td>8</td>
</tr>
</tbody>
</table>

## Overview

<table>
<thead>
<tr>
<th>Product name</th>
<th>Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade</th>
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<tbody>
<tr>
<td>Description</td>
<td>Rabbit polyclonal to Histone H4 (mono methyl K20) - ChIP Grade</td>
</tr>
<tr>
<td>Host species</td>
<td>Rabbit</td>
</tr>
<tr>
<td>Tested applications</td>
<td>Suitable for: IHC-P, IHC-Fr, WB, ICC/IF, Flow Cyt, ChIPseq, ChIP</td>
</tr>
<tr>
<td>Species reactivity</td>
<td>Reacts with: Mouse, Cow, Human, Caenorhabditis elegans, Drosophila melanogaster, Schizosaccharomyces pombe</td>
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<tr>
<td></td>
<td>Predicted to work with: Mammal</td>
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</table>

### Immunogen

Synthetic peptide within Human Histone H4 aa 1-100 (mono methyl K20) conjugated to Keyhole Limpet Haemocyanin (KLH). The exact sequence is proprietary.

(Peptide available as ab17043)

### Positive control

WB: Calf thymus histone preparation and HeLa whole cell extract. IHC-P: Human normal skin tissue.

### General notes

Interphase: The cells within the culture show a considerable variability in the intensity of staining with the antibody. The relationship between trimethylation levels and cell cycle have not yet been determined but may be a contributor to the amount of methylation detected in each cell.

Heterochromatic regions of the interphase nucleus are the primary sites of trimethylation observed by indirect immunofluorescence. Mitosis: Discrete chromosomal regions are labelled intensely, with lower level fluorescence throughout the remainder of the chromosome arms. Data from IF performed by Kirk McManus in the lab of Michael Hendzel as part of the antibody characterisation at cellnucleus.com

## Properties

<table>
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<tr>
<th>Form</th>
<th>Liquid</th>
</tr>
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<tbody>
<tr>
<td>Storage instructions</td>
<td>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.</td>
</tr>
</tbody>
</table>
| 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**
Interphase: The cells within the culture show a considerable variability in the intensity of staining with the antibody. The relationship between trimethylation levels and cell cycle have not yet been determined but may be a contributor to the amount of methylation detected in each cell. Heterochromatic regions of the interphase nucleus are the primary sites of trimethylation observed by indirect immunofluorescence. Mitosis: Discrete chromosomal regions are labelled intensely, with lower level fluorescence throughout the remainder of the chromosome arms. Data from IF performed by Kirk McManus in the lab of Michael Hendzel as part of the antibody characterisation at cellnucleus.com

**Clonality**
Polyclonal

**Isotype**
IgG

**Applications**

Our Abpromise guarantee covers the use of ab9051 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

<table>
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<tr>
<th>Application</th>
<th>Abreviews</th>
<th>Notes</th>
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<tbody>
<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 0.2 µg/ml.</td>
</tr>
<tr>
<td>IHC-Fr</td>
<td></td>
<td>Use at an assay dependent concentration.</td>
</tr>
<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000. Can be blocked with Human Histone H4 (mono methyl K20) peptide (ab17043).</td>
</tr>
<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration. Cautionary note: At low dilutions, the antibody may cross-react with microtubules. This can be minimized by increasing the dilution of the antibody when used in immunofluorescence.</td>
</tr>
<tr>
<td>Flow Cyt</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/200.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ab171870 - Rabbit polyclonal IgG, is suitable for use as an isotype control with this antibody.</td>
</tr>
<tr>
<td>CHIPseq</td>
<td></td>
<td>Use at an assay dependent concentration. PubMed: 22196736</td>
</tr>
<tr>
<td>ChIP</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use 2-25 µg for 25 µg of chromatin.</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 H4 family.

**Post-translational**
Acetylation at Lys-6 (H4K5ac), Lys-9 (H4K8ac), Lys-13 (H4K12ac) and Lys-17 (H4K16ac)
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. Phosphorylated by PAK2 at Ser-48 (H4S47ph). This phosphorylation increases the association of H3.3-H4 with the histone chaperone HIRA, thus promoting nucleosome assembly of H3.3-H4 and inhibiting nucleosome assembly of H3.1-H4.

Cellular localization

Nucleus. Chromosome.

Chromatin was prepared from U2OS cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 2 µg of ab9051 (blue), and 20 µl 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.

All lanes: Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051) at 1 µg/ml

Lane 1: Calf Thymus Histone Preparation
Western blot - Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051)

Lane 2 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H4 peptide (ab14963) at 0.5 µg/ml

Lane 3 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H3 (mono methyl K4) peptide (ab1340) at 0.5 µg/ml

Lane 4 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H3 (di methyl K4) peptide (ab7768) at 0.5 µg/ml

Lane 5 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K4) peptide (ab1342) at 0.5 µg/ml

Lane 6 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H4 (mono methyl K20) peptide (ab17043) at 0.5 µg/ml

Lane 7 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Histone H4 peptide (17-24) - di methyl K20 at 0.5 µg/ml

Lane 8 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H4 (tri methyl K20) peptide (ab17567) at 0.5 µg/ml

Lane 9 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H3 (mono methyl K9) peptide (ab1771) at 0.5 µg/ml

Lane 10 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H3 (di methyl K9) peptide (ab1772) at 0.5 µg/ml

Lane 11 : Calf Thymus Histone Preparation
Nuclear Lysate (ab121) with Human Histone H3 (tri methyl K9) peptide (ab1773) at 0.5 µg/ml

Lysates/proteins at 0.5 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution
Performed under reducing conditions.

**Observed band size**: 13 kDa

**Exposure time**: 10 seconds

Rabbit polyclonal to Histone H4 mono methyl K20 (1/250).

HeLa cells cultured on glass coverslips were fixed with 4% paraformaldehyde and then stained with ab9051 (green). Total chromatin was visualized using DAPI staining (red).

This image is part of the antibody characterisation at www.cellnucleus.com
IHC image of Histone H4 (mono methyl K20) staining in Human normal skin formalin fixed paraffin embedded tissue section*, performed on a Leica BondTM 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 ab9051, 0.2µg/ml, for 15 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.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre

IHC - Wholmount of Caenorhabditis elegans embryo labelling Histone H4 (mono methyl K20) with ab9051. Sample was incubated with primary antibody (1/400 in TBS + 0.1% Triton X-100 + 3% Goat serum) for 1 hour at 22°C. An Alexa Fluor® 488-conjugated Goat anti-rabbit IgG polyclonal (1/1000) was used as the secondary antibody. Left - Histone H4 (mono methyl K20), Middle - DAPI, Right - merge.

This image is courtesy of an anonymous Abreview
Western blot - Anti-Histone H4 (mono methyl K20)
antibody - ChIP Grade (ab9051)

Rabbit polyclonal to Histone H4 mono methyl K20 at 1/1000 on histone prep (Sigma).

Peptide competition (shown above lanes) at 1ug/ml.

This peptide blocking study shows that ab9051 is specific for Histone H4 (mono methyl K20). The activity of the antibody is specifically blocked by the addition of the H4 mono methyl peptide (ab17043).

Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (mono methyl K20) antibody - ChIP Grade (ab9051)

This image is courtesy of Kirk McManus in the lab of Michael Hendzel, University of Alberta

Rabbit polyclonal to Histone H4 mono methyl K20 (1/250).

HeLa cells cultured on glass coverslips were fixed with 4% paraformaldehyde and then stained with ab9051 (green). Total chromatin was visualized using DAPI staining (red).

Left: merge; center: DAPI; right: Mono K20

This image is part of the antibody characterisation at www.cellnucleus.com
Rabbit polyclonal to Histone H4 mono methyl K20 (1/250).

10T1 cells cultured on glass coverslips were fixed with 4% paraformaldehyde and then stained with ab9051.

Top left: metaphase cell. Mono methyl K20 (green); DAPI (red)
Top right: interphase cell. Mono methyl K20 (green); DAPI (red)
Lower left: interphase cell. Mono methyl K20 (green); DAPI (blue), TBP (red)
Lower right: interphase cell. Mono methyl K20 (green); DAPI (red)

This image is part of the antibody characterisation at www.cellnucleus.com

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