# abcam

# Product datasheet

# Anti-Histone H4 (di methyl K20) antibody ab9052

★★★★★ 11 Abreviews 52 References 5 Images

Overview

Product name Anti-Histone H4 (di methyl K20) antibody

**Description** Rabbit polyclonal to Histone H4 (di methyl K20)

Host species Rabbit

Tested applications Suitable for: IP, WB, ChIP, ICC/IF, IHC-P

**Species reactivity** Reacts with: Mouse, Cow, Human, Drosophila melanogaster, Schizosaccharomyces pombe,

Toxoplasma gondii

Predicted to work with: Rat, a wide range of other species 
• Does not react with:

Saccharomyces cerevisiae

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

Positive control WB: Calf thymus histone preparation and HeLa whole cell extract and S. pombe. IHC-P: FFPE

human pancreas adenocarcinoma tissue. ICC/IF: HeLa cell line

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.

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**Purity** Immunogen affinity purified

**Clonality** Polyclonal

**Isotype** IgG

#### **Applications**

# The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab9052 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
IP		Use at an assay dependent concentration.
WB	<b>★★★★</b> (5)	1/2000. Detects a band of approximately 11 kDa.
ChIP	*** <u>*</u>	Use at an assay dependent concentration. PubMed: 20829797
ICC/IF	*** <u>*</u>	Use a concentration of 1 µg/ml.
IHC-P	<b>★★★★★ (2)</b>	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

#### **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 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 PAD4 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.

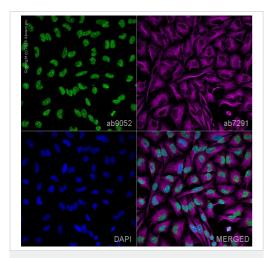
Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage. The exact role of H4K91ub1 in 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.

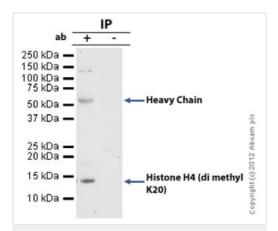
### **Images**



Immunocytochemistry/ Immunofluorescence - Anti-Histone H4 (di methyl K20) antibody (ab9052)

ab9052 staining Histone H4 (di methyl K20) in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab9052 at 0.1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin-Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour magenta). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 4% paraformaldehyde (10 min). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunoprecipitation - Anti-Histone H4 (di methyl K20) antibody (ab9052)

Histone H4 (di methyl K20) was immunoprecipitated using 0.5mg HeLa whole cell extract,  $5\mu g$  of Rabbit polyclonal to Histone H4 (di methyl K20) and  $50\mu l$  of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Hela whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab9052.

Secondary: Clean blot (HRP conjugate) at 1/1000 dilution.

Band: 14kDa: Histone H4 (di methyl K20).

1 2 3 4 5 H4 Di MeK20→

Western blot - Anti-Histone H4 (di methyl K20) antibody (ab9052)

This image is courtesy of Steve Sanders, Tony Kouzarides lab, University of Cambridge

**All lanes :** Anti-Histone H4 (di methyl K20) antibody (ab9052) at 1/2000 dilution

Lane 1: yH4 17-24 peptide

Lane 2: Unmodified yH4 17-24 peptide

Lane 3: Mono methyl K20 yH4 17-24 peptide

Lane 4: Di methyl K20 yH4 17-24 peptide

Lane 5: Tri methyl K20 yH4 17-24 peptide

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Blocking peptides at 1 µg/ml per lane.

Performed under reducing conditions.

All lanes: Upper blot - Histone H4 antibody (gift of A. Verreault)

Lower blot - Rabbit polyclonal to Histone H4 di methyl K20 (ab9052) at 1/2000

Lane 1: S.cerevisiae extract

Lane 2: S.pombe extract

Lane 3: S.pombe rH4

Lane 4: Drosophila rH4

Lane 5: Histones (Roche)

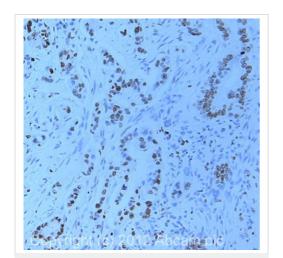
Di methylation at K20 is seen in S. pombe and in calf thymus (Roche). S. cerevisiae lacks K20 (di) methylation.

IHC image of ab9052 staining in human pancreas adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond TM 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 ab9052, 1µg/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.



Western blot - Anti-Histone H4 (di methyl K20) antibody (ab9052)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H4 (di methyl K20) antibody (ab9052)

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