

Product datasheet

Anti-Histone H4 (di methyl K20) antibody ab9052

★★★★☆ 11 Abreviews 46 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	<p>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.</p> <p>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</p>

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 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	★★★★★ (5)	1/2000. Detects a band of approximately 11 kDa.
ChIP	★★★★★ (1)	Use at an assay dependent concentration. PubMed: 20829797
ICC/IF	★★★★★ (2)	Use a concentration of 1 µg/ml.
IHC-P	★★★★★ (2)	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.

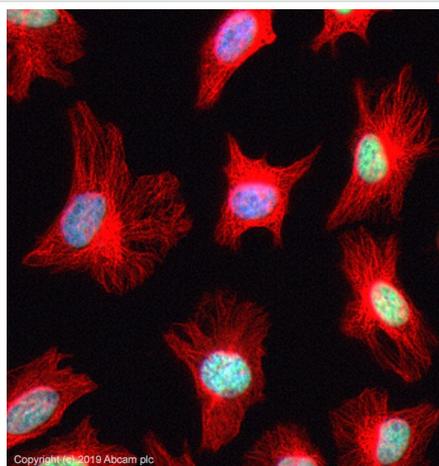
Ubiquitinated by the CUL4-DDB-RBX1 complex in response to ultraviolet irradiation. This may weaken the interaction between histones and DNA and facilitate DNA accessibility to repair proteins. Monoubiquitinated at Lys-92 of histone H4 (H4K91ub1) in response to DNA damage.

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).

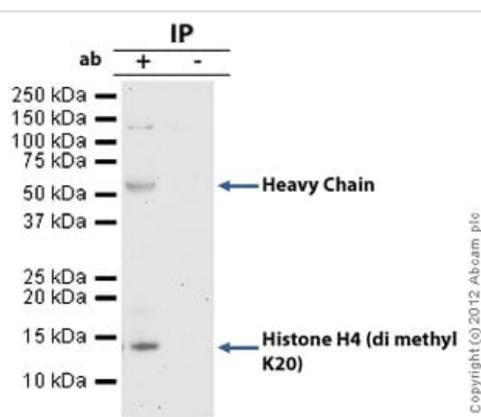
Sumoylated, which is associated with transcriptional repression.

Cellular localization Nucleus. Chromosome.

Images



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



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



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

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

ab9052 stained in HeLa cells. Cells were fixed with 4% paraformaldehyde (10 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 ab9052 at 0.5µg/ml and ab7291 (Mouse monoclonal to alpha Tubulin - Loading Control) used at a 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150081, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed, (pseudo-colored green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594) preadsorbed, (colored red), both used at a 1/1000 dilution 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.

Histone H4 (di methyl K20) was immunoprecipitated using 0.5mg HeLa whole cell extract, 5µg of Rabbit polyclonal to Histone H4 (di methyl K20) and 50µl 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).

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

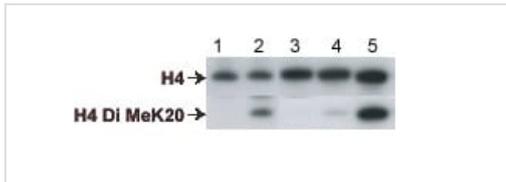
Lane 3 : Mono methyl K20 yH4 17-24 peptide

Lane 4 : Di methyl K20 yH4 17-24 peptide

Lane 5 : Tri methyl K20 yH4 17-24 peptide

Blocking peptides at 1 µg/ml per lane.

Performed under reducing conditions.



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

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

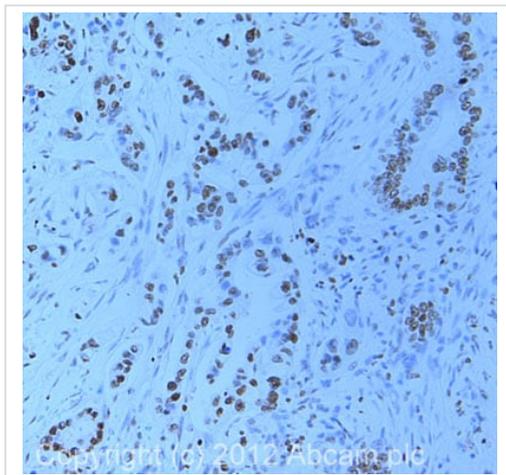
Lane 2 : *S.pombe* extract

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.



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

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

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