abcam

Product datasheet

Anti-Histone H4 (tri methyl K20) antibody - ChIP Grade ab9053

19 Abreviews 193 References 5 Images

Overview

Product name	Anti-Histone H4 (tri methyl K20) antibody - ChIP Grade
Description	Rabbit polyclonal to Histone H4 (tri methyl K20) - ChIP Grade
Host species	Rabbit
Specificity	Histone H4 tri-methylated at Lys 20.
Tested applications	**Suitable for:** IHC-P, WB, ChIP
Species reactivity	**Reacts with:** Cow, Human, Recombinant fragment **Predicted to work with:** Mouse, Rat, Saccharomyces cerevisiae, Schizosaccharomyces pombe, Mammals, Toxoplasma gondii, Xenopus tropicalis
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab17567**)
General notes	Mouse: Prominent staining of a subset of centromeric or pericentromeric heterochromatin. 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 immunofluroescence. Mitosis: Discrete chromosomal regions are labelled intensely, with lower level fluorescence throughout the remainder of the chromosome arms. Immunofluorescence staining was performed as part of the nuclear antibody characterisation program at www.cellnucleus.com. Trimenthylation at Lys20 of Histone H4 isa common hallmark of human cancer (Fraga et al. 2005). 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.
Purity	Immunogen affinity purified
Purification notes	Purified using Sulpholink column with specific peptide linked via its cysteine residue
Primary antibody notes	In immunofluorescence: Human cells: The antibody stains a subset of cellular chromatin that is characteristically DAPI-rich (condensed, heterochromatic). The perinucleolar heterochromatin is particularly rich in trimethylated lysine 20 staining. Mouse: Prominent staining of a subset of centromeric or pericentromeric heterochromatin. 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 immunofluroescence. Mitosis: Discrete chromosomal regions are labelled intensely, with lower level fluorescence throughout the remainder of the chromosome arms. Immunofluorescence staining was performed as part of the nuclear antibody characterisation program at www.cellnucleus.com. Trimenthylation at Lys20 of Histone H4 isa common hallmark of human cancer (Fraga et al. 2005).
Clonality	Polyclonal
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab9053 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

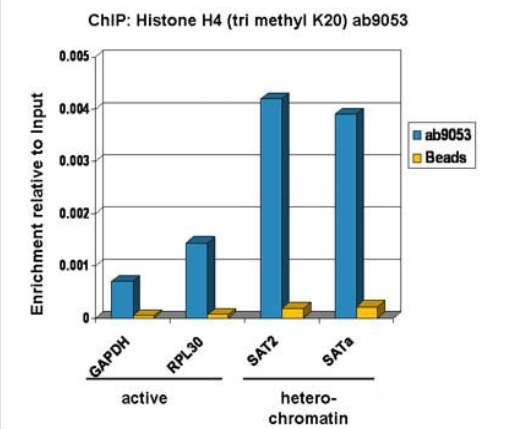
Application	Abreviews	Notes
IHC-P	★★★★★ **(2)**	Use at an assay dependent concentration.
WB	★★★★☆ **(9)**	Use a concentration of 1 µg/ml. Detects a band of approximately 13 kDa (predicted molecular weight: 11 kDa).
ChIP	★★★★☆ **(4)**	Use 4-5µg for 10^6 cells.

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

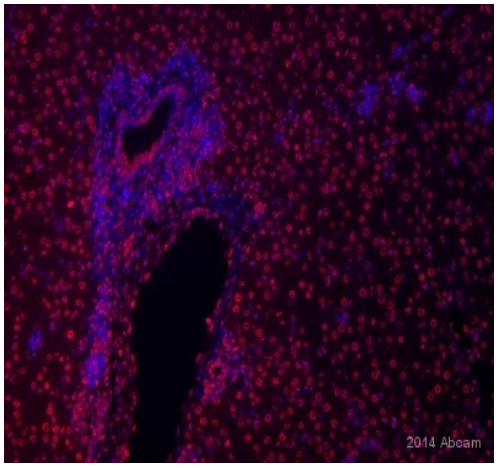
	histones, also called histone code, and nucleosome remodeling.
Sequence similarities	Belongs to the histone H4 family.
Post-translational modifications	Acetylation at Lys-6 (H4K5ac), Lys-9 (H4K8ac), Lys-13 (H4K12ac) and Lys-17 (H4K16ac) occurs in coding regions of the genome but not in heterochromatin. Citrullination at Arg-4 (H4R3ci) by 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.

Images



ChIP - Anti-Histone H4 (tri methyl K20) antibody - ChIP Grade (ab9053)

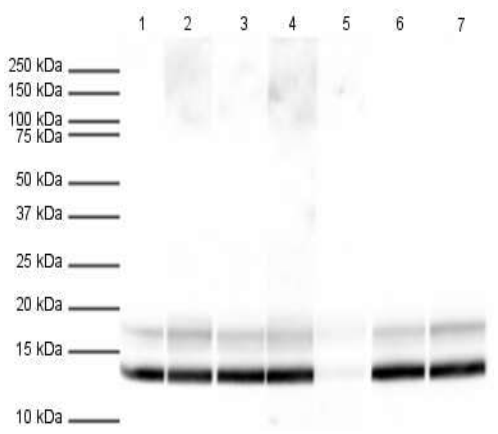
Chromatin was prepared from U2OS cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 6 µl of ab9053 (blue), and 20 µl of protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach). Primers and probes are located in the first kb of the transcribed region.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (tri methyl K20) antibody - ChIP Grade (ab9053)

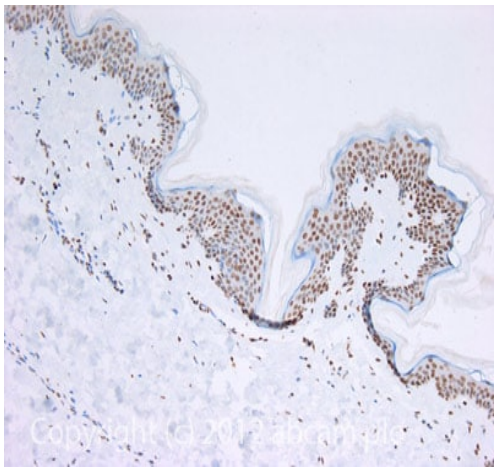
This image is courtesy of an anonymous Abreview.

Immunohistochemical staining of paraffin embedded, PFA fixed, mouse liver using ab9053 at 1/100. Heat mediated antigen retrieval was performed using sodium citrate buffer, pH 6, and the sample was permeabilized with 0.05% Triton X 100. The sample was blocked in 5% BSA for 1 hour at room temperature and was then incubated with primary antibody for 3 hours in PBS/0.05% Triton X 100 at room temperature. An Alexa Fluor® 555 donkey anyti-rabbit was used as the secondary at 1/250.



Western blot - Anti-Histone H4 (tri methyl K20) antibody - ChIP Grade (ab9053)

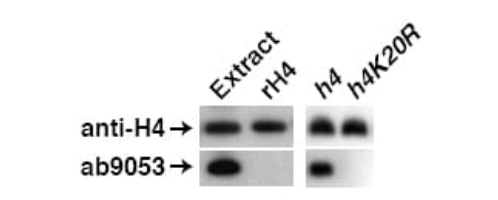
ab9053 is specific for Histone H4 (tri-methyl K20). This is illustrated in lane 5 where the activity of ab9053 is specifically blocked by the addition of the immunizing peptide (**ab17567**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H4 (tri methyl K20) antibody - ChIP Grade (ab9053)

IHC image of Histone H4 (tri 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 ab9053, 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.



Western blot - Anti-Histone H4 (tri methyl K20) antibody - ChIP Grade (ab9053)

Left panel: a fission yeast whole cell extract and recombinant (r) fission yeast histone H4.

Right panel: fission yeast whole cell extracts from strains with only a single wt (h4) or mutant (h4K20R) gene were immunoblotted.

Review by Steven Sanders submitted 13 August 2004

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