abcam

Product datasheet

Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified ab195497

6 Images

Overview

Product name	Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified
Description	Rabbit polyclonal to Histone H3 (tri methyl K9) - ChIP Grade, purified
Host species	Rabbit
Tested applications	**Suitable for:** Dot blot, ChIP, WB, ICC/IF, ChIP-sequencing
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide corresponding to Human Histone H3 (tri methyl K9) conjugated to keyhole limpet haemocyanin.
Positive control	ICC/IF: HeLa cells. ChIP: HeLa cells. WB: Histone extracts from HeLa cells.
General notes	Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough. Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility. We are also planning to innovate the way in which we present recommended applications and species on our product datasheets, so that only applications & species that have been tested in our own labs, our suppliers or by selected trusted collaborators are covered by our Abpromise™ guarantee. In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for. We are also updating the applications & species that this product has been "predicted to work with," however this information is not covered by our Abpromise guarantee. Applications & species from publications and Abreviews that have not been tested in our own labs or in those of our suppliers are not covered by the Abpromise guarantee. 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, as well as 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 long term. Avoid freeze / thaw cycle.
Storage buffer	Preservatives: 0.05% Sodium azide, 0.05% Proclin 300 Constituent: 99% PBS
Purity	Protein A purified
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab195497** 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
Dot blot		1/20000.
ChIP		Use at an assay dependent concentration. 1-5 µg/ChIP
WB		1/1000. Predicted molecular weight: 15 kDa.
ICC/IF		1/500.
ChIP-sequencing		Use at an assay dependent concentration. 1-5 µg/ChIP

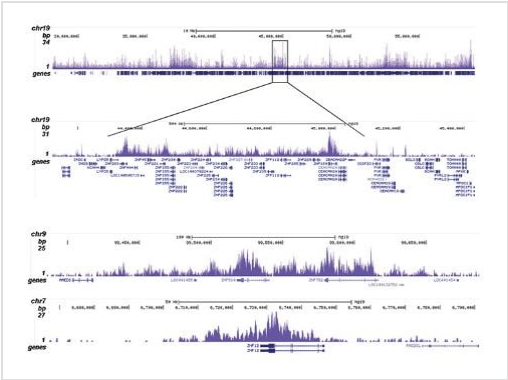
Target

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 H3 family.
Developmental stage	Expressed during S phase, then expression strongly decreases as cell division slows down during the process of differentiation.
Post-translational modifications	Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs methylation at Arg-9 (H3R8me2s). Acetylation on Lys-19 (H3K18ac) and Lys-24 (H3K24ac) favors methylation at Arg-18 (H3R17me). Citrullination at Arg-9 (H3R8ci) and/or Arg-18 (H3R17ci) by PADI4 impairs methylation and represses transcription. Asymmetric dimethylation at Arg-18 (H3R17me2a) by CARM1 is linked to gene activation. Symmetric dimethylation at Arg-9 (H3R8me2s) by PRMT5 is linked to gene repression. Asymmetric dimethylation at Arg-3 (H3R2me2a) by PRMT6 is linked to gene repression and is mutually exclusive with H3 Lys-5 methylation (H3K4me2 and H3K4me3). H3R2me2a is present at

the 3' of genes regardless of their transcription state and is enriched on inactive promoters, while it is absent on active promoters.
Methylation at Lys-5 (H3K4me), Lys-37 (H3K36me) and Lys-80 (H3K79me) are linked to gene activation. Methylation at Lys-5 (H3K4me) facilitates subsequent acetylation of H3 and H4. Methylation at Lys-80 (H3K79me) is associated with DNA double-strand break (DSB) responses and is a specific target for TP53BP1. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are linked to gene repression. Methylation at Lys-10 (H3K9me) is a specific target for HP1 proteins (CBX1, CBX3 and CBX5) and prevents subsequent phosphorylation at Ser-11 (H3S10ph) and acetylation of H3 and H4. Methylation at Lys-5 (H3K4me) and Lys-80 (H3K79me) require preliminary monoubiquitination of H2B at 'Lys-120'. Methylation at Lys-10 (H3K9me) and Lys-28 (H3K27me) are enriched in inactive X chromosome chromatin.
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes following external stimulation, like mitogens, stress, growth factors or UV irradiation and result in the activation of genes, such as c-fos and c-jun. Phosphorylation at Ser-11 (H3S10ph), which is linked to gene activation, prevents methylation at Lys-10 (H3K9me) but facilitates acetylation of H3 and H4. Phosphorylation at Ser-11 (H3S10ph) by AURKB mediates the dissociation of HP1 proteins (CBX1, CBX3 and CBX5) from heterochromatin. Phosphorylation at Ser-11 (H3S10ph) is also an essential regulatory mechanism for neoplastic cell transformation. Phosphorylated at Ser-29 (H3S28ph) by MLTK isoform 1, RPS6KA5 or AURKB during mitosis or upon ultraviolet B irradiation. Phosphorylation at Thr-7 (H3T6ph) by PRKCBB is a specific tag for epigenetic transcriptional activation that prevents demethylation of Lys-5 (H3K4me) by LSD1/KDM1A. At centromeres, specifically phosphorylated at Thr-12 (H3T11ph) from prophase to early anaphase, by DAPK3 and PKN1. Phosphorylation at Thr-12 (H3T11ph) by PKN1 is a specific tag for epigenetic transcriptional activation that promotes demethylation of Lys-10 (H3K9me) by KDM4C/JMJD2C.
Phosphorylation at Tyr-42 (H3Y41ph) by JAK2 promotes exclusion of CBX5 (HP1 alpha) from chromatin.
Monoubiquitinated by RAG1 in lymphoid cells, monoubiquitination is required for V(D)J recombination (By similarity). 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.

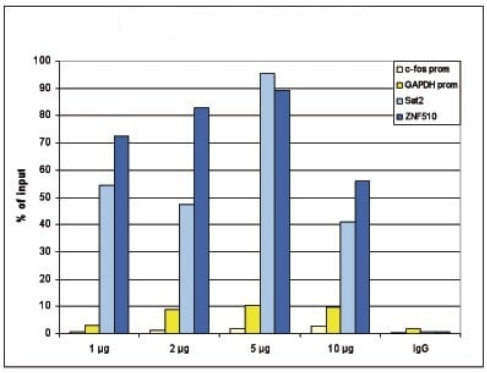
Cellular localization Nucleus. Chromosome.

Images



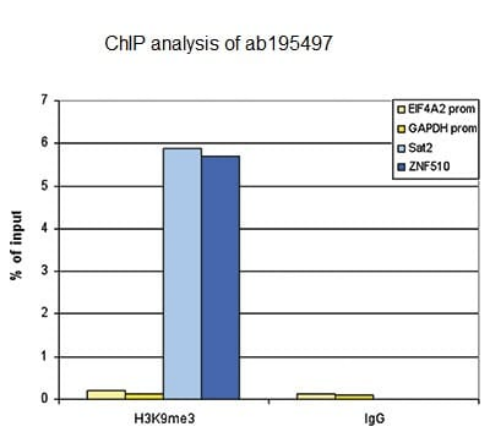
ChIP-sequencing - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified (ab195497)

ChIP-seq analysis using Human HeLa cells, labeling Histone H3 (tri methyl K9) with ab195497 at 0.5 µg and optimized PCR primer sets for qPCR as described in above. The 36 bp tags were aligned to the human genome using the BWA algorithm. The peak distribution is shown along the long arm of chromosome 19 and a zoomin to an enriched region containing several ZNF repeat genes (A and B) and the enrichment at ZNF510 and ZNF12 on chromosome 9 and 7 respectively (C and D).



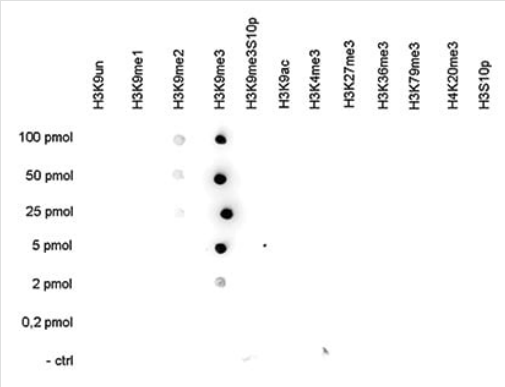
ChIP - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified (ab195497)

ChIP analysis using Human HeLa cells, labeling Histone H3 (tri methyl K9) with ab195497 and optimized PCR primer sets for qPCR. ChIP was performed using sheared chromatin from 1000,000 cells. A titration of the antibody consisting of 1, 2, 5 and 10 µg per ChIP experiment was analysed. IgG (2 µg/IP) was used as negative IP control. QPCR was performed with primers for the heterochromatin marker Sat2 and for the ZNF510 gene, used as positive controls, and for the promoters of the active c-fos and GAPDH genes, used as negative controls. The graph shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



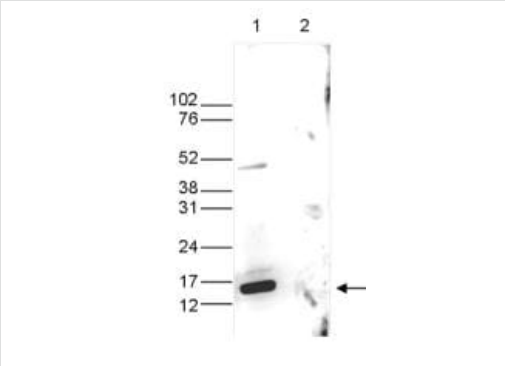
ChIP - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified (ab195497)

ChIP analysis using HeLa cells, labeling Histone H3 (tri methyl K27) with ab195497. ChIP was performed using sheared chromatin from 1000,000 cells. The IP'd DNA was analysed by QPCR with optimized PCR primer pairs for the promoter regions of the active GAPDH and EIF4A2 genes, for the coding region of the ZNF510 gene and for the Sat2 satellite repeat. The graph shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



Dot Blot - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified (ab195497)

Dot Blot analysis of peptides containing modified and unmodified sequences of histone H3 and H4, labeling Histone H3 (tri methyl K9) with ab195497 at 1/20000 dilution. 0.2-100 pmol of the peptide containing the respective histone modification were spotted onto the membrane.



Western blot - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified (ab195497)

All lanes : Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified (ab195497) at 1/1000 dilution (in TBS-Tween containing 5% skimmed milk)

Lane 1 : Histone extracts from HeLa cells at 15 µg
Lane 2 : Recombinant H3 at 1 µg

Predicted band size: 15 kDa

Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K9) antibody - ChIP Grade, purified (ab195497)

Immunofluorescent analysis of HeLa cells labeling Histone H3 (tri methyl K9) with ab195497 at 1/500 dilution in blocking solution followed by an anti-rabbit antibody conjugated to Alexa488 (left), DAPI (center) or a merge of the two stainings (right). Cells were fixed with 4% formaldehyde for 10 minutes and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery

- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors