


## Product datasheet

# Anti-Histone H3 (tri methyl K27) antibody - ChIP Grade ab195477

[9 References](#) [5 Images](#)

### Overview

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<b>Product name</b>	Anti-Histone H3 (tri methyl K27) antibody - ChIP Grade
<b>Description</b>	Rabbit polyclonal to Histone H3 (tri methyl K27) - ChIP Grade
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> WB, ICC/IF, Dot blot, ChIP, ChIP-sequencing
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Human, Recombinant fragment <b>Predicted to work with:</b> Rat, Arabidopsis thaliana, Drosophila melanogaster, Zebrafish, Schistosoma japonicum 
<b>Immunogen</b>	Synthetic peptide corresponding to Human Histone H3 (tri methyl K27) conjugated to keyhole limpet haemocyanin.
<b>Positive control</b>	Chromatin prepared from HeLa cells; Chromatin prepared from HeLaS3 cells; HeLa whole cell extract; HeLa histone extract; NIH 3T3 cells.
<b>General notes</b>	<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&amp;As</p>

### Properties

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<b>Form</b>	Liquid
<b>Storage instructions</b>	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.
<b>Storage buffer</b>	Preservatives: 0.05% Sodium azide, 0.05% Proclin 300 Constituent: 99% PBS
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Polyclonal
<b>Isotype</b>	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab195477 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
WB		1/500. Predicted molecular weight: 15 kDa.
ICC/IF		1/200.
Dot blot		1/5000.
ChIP		Use 1µg for 10 <sup>6</sup> cells.
ChIP-sequencing		Use 1µg for 10 <sup>6</sup> 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 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

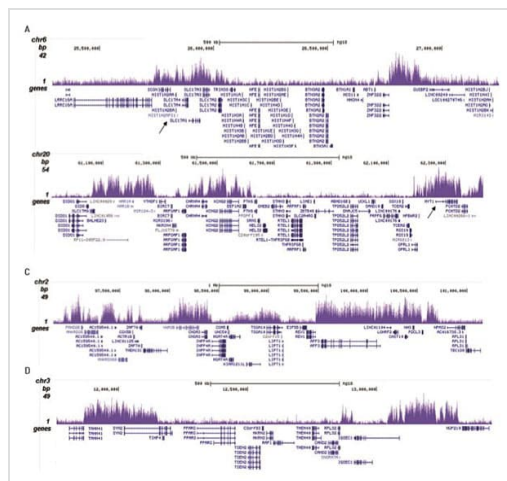
during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and RPS6KA5 is important during interphase because it enables the transcription of genes 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.

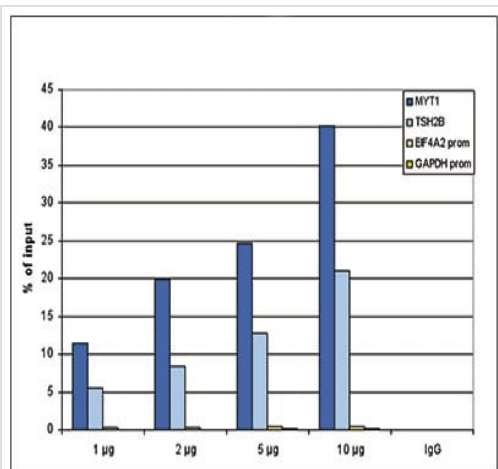
**Images**



ChIP-sequencing - Anti-Histone H3 (tri methyl K27) antibody - ChIP Grade (ab195477)

ChIPseq results obtained with ab195477 directed against Histone H3 (tri methyl K27).

ChIP was performed on sheared chromatin from 1 million HeLaS3 cells using 1 µg of ab195477. The IP'd DNA was subsequently analysed on an Illumina HiSeq. Library preparation, cluster generation and sequencing were performed according to the manufacturer's instructions. The 51 bp tags were aligned to the human genome using the BWA algorithm. Figure shows the enrichment in genomic regions of chromosome 6, surrounding the TSH2B gene (indicated by an arrow; fig A), of chromosome 20, surrounding the MYT1 gene (fig B), and of chromosome 2 and 3 (figure C and D).



ChIP - Anti-Histone H3 (tri methyl K27) antibody - ChIP Grade (ab195477)

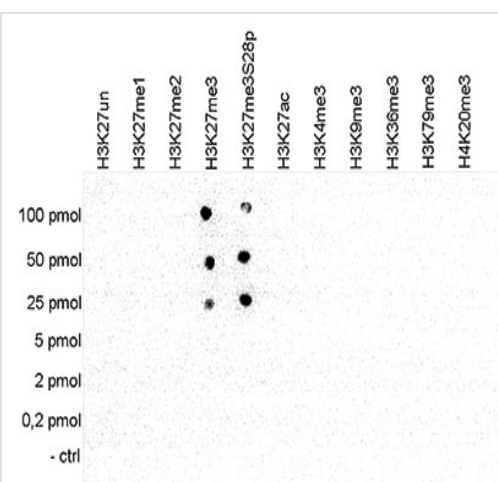
ChIP results obtained with ab195477 directed against Histone H3 (tri methyl K27).

ChIP assays were performed using human HeLa cells, ab195477 and optimized PCR primer sets for qPCR. ChIP was performed using sheared chromatin from 1 million 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 promoters of the active genes EIF4A2 and GAPDH as negative controls, and for the coding regions of the inactive genes MYT1 and TSH2B as positive controls. Figure shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis). These results are in accordance with the observation that H3K27me3 is preferably present at inactive genes.



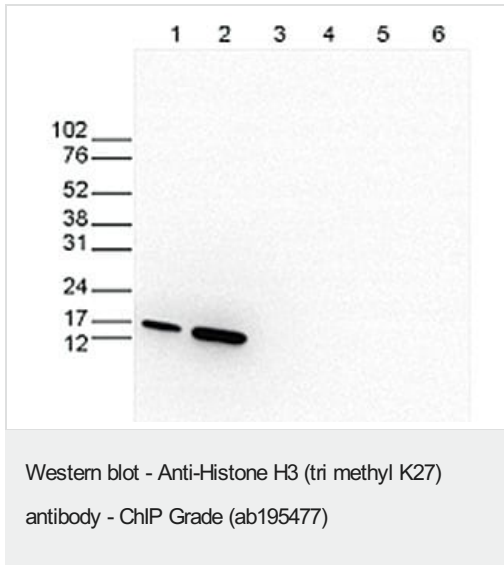
Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K27) antibody - ChIP Grade (ab195477)

Immunofluorescent analysis of NIH 3T3 cells labeling Histone H3 (tri methyl K27) with ab195477 at 1/200 dilution. Cells were fixed with 4% formaldehyde for 10 minutes and blocked with PBS/TX-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with ab195477 in blocking solution followed by an ant-rabbit antibody conjugated to Alexa488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.



Dot Blot - Anti-Histone H3 (tri methyl K27) antibody - ChIP Grade (ab195477)

Dot Blot analysis was performed to test the cross reactivity of ab195477 against Histone H3 (tri methyl K27) with peptides containing other modifications of Histone H3 and H4 and the unmodified H3K27 sequence. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1/5000. Figure shows a high specificity of the antibody for the modification of interest. Please note that that antibody also recognizes the modification if S28 is phosphorylated.



**All lanes :** Anti-Histone H3 (tri methyl K27) antibody - ChIP Grade (ab195477) at 1/500 dilution

**Lane 1 :** HeLa whole cell extract at 25 µg

**Lane 2 :** HeLa histone extract at 15 µg

**Lane 3 :** Recombinant Histone H2A at 1 µg

**Lane 4 :** Recombinant Histone H2B at 1 µg

**Lane 5 :** Recombinant Histone H3 at 1 µg

**Lane 6 :** Recombinant Histone H4 at 1 µg

**Predicted band size:** 15 kDa

The antibody was diluted in TBS-Tween containing 5% skimmed milk.

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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