abcam

Product datasheet

Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade ab6002

72 Abreviews 908 References 8 Images

Overview

Product name	Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade
Description	Mouse monoclonal [mAbcam 6002] to Histone H3 (tri methyl K27) - ChIP Grade
Host species	Mouse
Specificity	This antibody is specific for histone H3 tri-methylated at K27. The antibody is blocked in Western blot by tri methyl K27 peptide and slightly by di methyl K27 peptide (there is <12% cross reactivity with di methyl K27 as determined by ELISA). It is not blocked by mono methyl K4, di methyl K4, tri methyl K4, mono methyl K9, di methyl K9, tri methyl K9, mono methyl K27 or unmodified K27 peptides (see the peptide blocking assay blot below).
Tested applications	**Suitable for:** ChIP, ELISA, WB, IHC - Wholemount, ICC/IF
Species reactivity	**Reacts with:** Mouse, Cow, Human, Recombinant fragment **Predicted to work with:** Rat, Rabbit, Chicken, Xenopus laevis, Arabidopsis thaliana, Drosophila melanogaster, Plants, Zebrafish, Rhesus monkey, Chinese hamster, Rice
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers. (Peptide available as **ab1782**)
Positive control	WB: Calf Thymus Histone Preparation Nuclear Lysate, HeLa cell lysates, mouse ES whole cell lysate ChIP: HeLa cells and K562 cells. ICC/IF: HeLa cells. IHC-Wholemount: blastocysts and germ cells and in culture in pluripotent stem cells. ELISA: Histone H3 - tri methyl K27 peptide.
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact **orders@abcam.com**. 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 Constituents: PBS, 6.97% L-Arginine
Purity	Protein A purified
Clonality	Monoclonal
Clone number	mAbcam 6002
Isotype	IgG3
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab6002 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
ChIP	★★★★★ **(19)**	Use 5-10 µg for 25 µg of chromatin. Use Myo-D ChIP primer pair **ab269261** as positive control.
ELISA		Use a concentration of 0.025 - 1 µg/ml.
WB	★★★★☆ **(21)**	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).Can be blocked with **Human Histone H3 (tri methyl K27) peptide (ab1782)**. Blocking: we recommend using 3% milk block for 1 hour at room temperature. This step may need to be optimized for your experiments.
IHC - Wholemount		Use at an assay dependent concentration.
ICC/IF	★★★★☆ **(17)**	Use a concentration of 5 µg/ml.

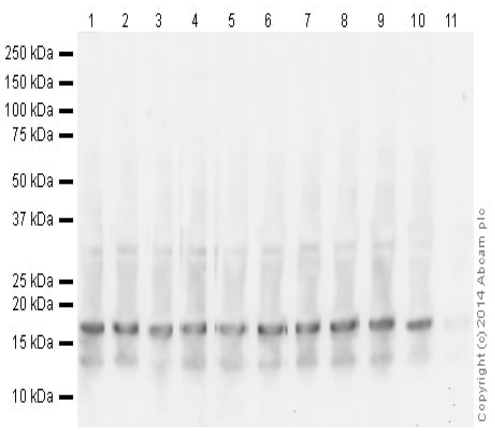
Target

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



Western blot - Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002)

All lanes : Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate
Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 peptide (**ab17163**) at 0.5 µg/ml
Lane 3 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K4) peptide (**ab1340**) at 0.5 µg/ml
Lane 4 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K4) peptide (**ab7768**) at 0.5 µg/ml
Lane 5 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K4) peptide (**ab1342**) at 0.5 µg/ml
Lane 6 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K9) peptide (**ab1771**) at 0.5 µg/ml
Lane 7 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K9) peptide (**ab1772**) at 0.5 µg/ml
Lane 8 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K9) peptide (**ab1773**) at 0.5 µg/ml
Lane 9 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (mono methyl K27) peptide (**ab1780**) at 0.5 µg/ml
Lane 10 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (di methyl K27) peptide (**ab1781**) at 0.5 µg/ml
Lane 11 : Calf Thymus Histone Preparation Nuclear Lysate with Human Histone H3 (tri methyl K27) peptide (**ab1782**) at 0.5 µg/ml

Lysates/proteins at 0.25 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/50000 dilution

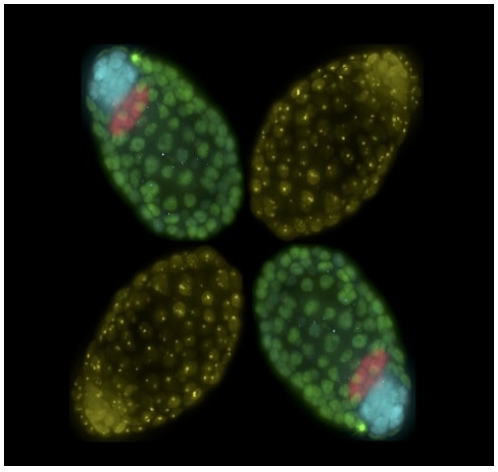
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 17 kDa

Exposure time: 3 minutes

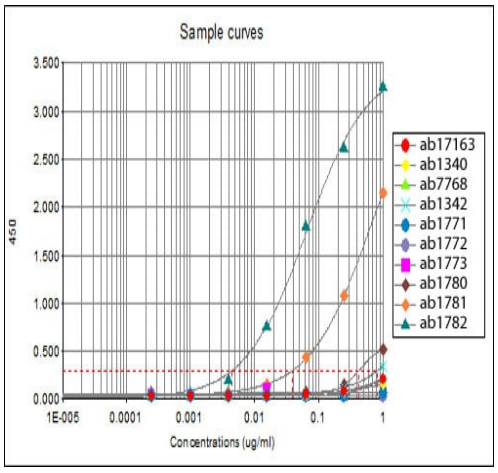
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab6002 overnight at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution **ab133406**.



IHC - Wholemount - Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002)

In mice, the X-inactivation process is reversed naturally by X-reactivation in blastocysts and germ cells and in culture in pluripotent stem cells. The image shows late blastocyst-stage mouse embryos consisting of three cell types: epiblast (NANOG-positive, cyan), primitive endoderm (GATA4-positive, red) and trophectoderm (CDX2-positive, green). The inactive X-chromosome (H3K27me3-positive, yellow dots) is reactivated only in the epiblast (cells without yellow spots), which will form the embryo. The germ cell factor PRDM14 and the long noncoding RNA Tsix collaborate during the X-reactivation process in blastocysts and pluripotent stem cells and thereby link epigenetic with cellular reprogramming events.

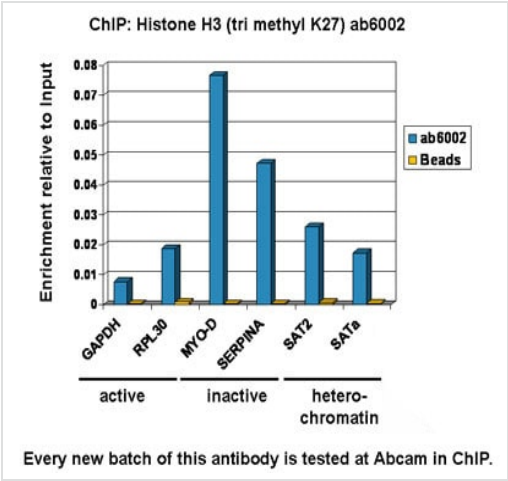
Image is courtesy of Bernhard Payer, runner-up of the immunofluorescence imaging competition 2017.



ELISA - Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002)

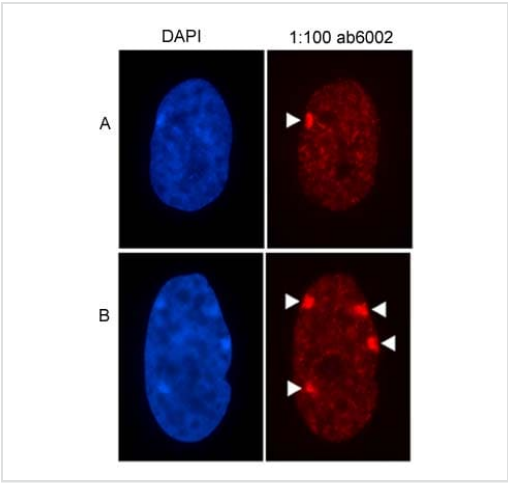
All batches of ab6002 are tested in ELISA against peptides to different Histone H3 modifications. Results show strong binding to Histone H3 - tri methyl K27 peptide (**ab1782**), indicating that this antibody specifically recognizes the Histone H3 tri methyl K27 modification. Weak binding is also detected against the Histone H3 di methyl K27 modification (<12%) (**ab1781**).

ab17163 - Histone H3 - unmodified
ab1340 - Histone H3 - mono methyl K4
ab7768 - Histone H3 - di methyl K4
ab1342 - Histone H3 - tri methyl K4
ab1771 - Histone H3 - mono methyl K9
ab1772 - Histone H3 - di methyl K9
ab1773 - Histone H3 - tri methyl K9
ab1780 - Histone H3 - mono methyl K27
ab1781 - Histone H3 - di methyl K27
ab1782 - Histone H3 - tri methyl K27



ChIP - Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002)

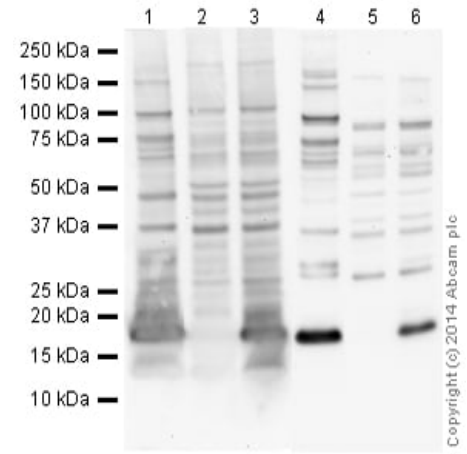
Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 min. The ChIP was performed with 25 µg of chromatin, 5 µg of ab6002 (blue), and 20 µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002)

Figure showing the nuclear distribution of H3 (tri-methyl K27) antibody, ab6002 in a) a 46 chromosome, XX cell line, and b) a 49 chromosome, XXXXX cell line.

The location of facultative heterochromatin at the inactive X chromosome is indicated by white arrow heads.



Western blot - Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002)

Lanes 1-3 : Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002) at 1 µg/ml (2% BSA)
Lanes 4-6 : Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002) at 1 µg/ml (3% MILK)

Lanes 1 & 4 : HeLa (Human epithelial carcinoma cell line) Nuclear Lysate
Lanes 2 & 5 : EED-/- mouse ES Whole Cell Lysate
Lanes 3 & 6 : WT mouse ES Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/50000 dilution

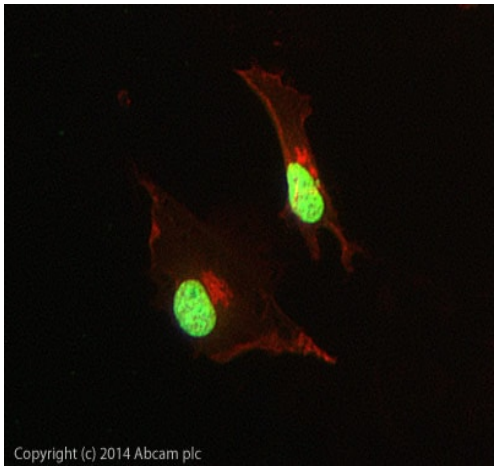
Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 15 kDa
Observed band size: 17 kDa

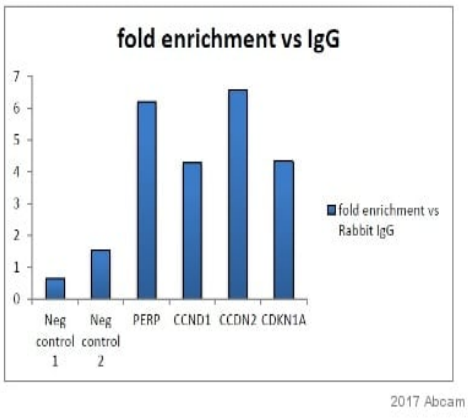
Exposure time: 12 minutes

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 2% Bovine Serum Albumin (lanes 1-3) and 3% milk (lanes 4-6) before being incubated with ab6002 overnight at 4°C. Antibody binding was detected using an anti-mouse antibody conjugated to HRP, and visualised using ECL development solution **ab133406**.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002)

ICC/IF image of ab6002 stained HeLa cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab6002, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-mouse IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in 4% PFA fixed (10 min) Hek293, HepG2 and MCF7 cells at 5µg/ml, and in 100% methanol fixed (5 min) HeLa, Hek293, HepG2 and MCF7 cells at 5µg/ml.



ChIP - Anti-Histone H3 (tri methyl K27) antibody [mAbcam 6002] - ChIP Grade (ab6002)

This image is courtesy of an anonymous abreview.

Chromatin was prepared from K562 cells. Cells were fixed with formaldehyde for 10 min. A GATA1 antibody was used as the positive control and a Rabbit IgG was used as the negative control. Incubation with primary antibody was in Immuno Precipitation Dilution buffer for 16 hours at 4°C. The immunoprecipitated DNA was quantified by real time PCR.

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