abcam

Product datasheet

Anti-Histone H3 antibody - Nuclear Marker and ChIP Grade ab 1791

*** * * 232 Abreviews 4330 References 14 Images

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Ove	rview	•

Product name Anti-Histone H3 antibody - Nuclear Marker and ChIP Grade

Description Rabbit polyclonal to Histone H3 - Nuclear Marker and ChIP Grade

Host species Rabbit

SpecificityBased only on sequence homology, we expect the antibody to react with multiple variants of H3

such as H3.1, H3.2 and H3.3.

Tested applications Suitable for: IHC-P, ChIP, IP, WB, ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human, Saccharomyces cerevisiae, Xenopus laevis, Arabidopsis

thaliana, Drosophila melanogaster, Indian muntjac, Schizosaccharomyces pombe

Predicted to work with: Chicken, Dog, Caenorhabditis elegans, Ferret, Zebrafish, a wide range of other species, Mammals, Silk worm, Dictyostelium discoideum, Rainbow trout, Neurospora crassa, Toxoplasma gondii, Rice, Schistosoma mansoni, Candida albicans, Cyanidioschyzon

merolae 📤

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

(Peptide available as ab12149)

General notes A recombinant rabbit monoclonal alternative is available to this target – <u>ab176842</u>

Rabbit polyclonal $\lg G$ ($\frac{ab171870}{}$) is suitable for use as an isotype control with this antibody.

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

1

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 Preservative: 0.02% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

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 ab1791 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	*** <u>(20)</u>	1/100 - 1/400. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.		
ChIP	★★★★★ (46)	Use 2µg for 10 ⁶ cells.		
IP	★★★★★ (4)	Use a concentration of 5 µg/ml.		
WB	**** (121)	1/1000 - 1/5000. Detects a band of approximately 17 kDa (predicted molecular weight: 15 kDa).Can be blocked with Human Histone H3 peptide (ab12149). We recommend Goat Anti-Rabbit IgG H&L (HRP) (ab6721) secondary antibody.		
ICC/IF	★★★★☆ (27)	Use a concentration of 1 µg/ml.		

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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

Acetylation is generally linked to gene activation. Acetylation on Lys-10 (H3K9ac) impairs

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 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 PAD4 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.

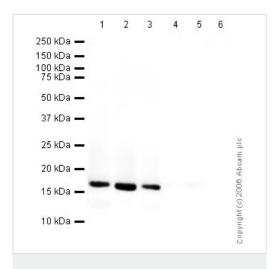
Phosphorylated at Thr-4 (H3T3ph) by GSG2/haspin during prophase and dephosphorylated during anaphase. Phosphorylation at Ser-11 (H3S10ph) by AURKB is crucial for chromosome condensation and cell-cycle progression during mitosis and meiosis. In addition phosphorylation at Ser-11 (H3S10ph) by RPS6KA4 and 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 antibody - Nuclear Marker and ChIP Grade (ab1791)

All lanes : Anti-Histone H3 antibody - Nuclear Marker and ChIP Grade (ab1791) at 1/1000 dilution

Lane 1 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate

Lane 3: HEK293 (Human embryonic kidney cell line) Whole Cell Lysate

Lane 4 : A431 (Human epithelial carcinoma cell line) Whole Cell Lysate with Human Histone H3 peptide (<u>ab12149</u>) at 1 μg/ml Lane 5 : Jurkat (Human T cell lymphoblast-like cell line) Whole Cell Lysate with Human Histone H3 peptide (<u>ab12149</u>) at 1 μg/ml Lane 6 : HEK293 (Human embryonic kidney cell line) Whole Cell Lysate with Human Histone H3 peptide (<u>ab12149</u>) at 1 μg/ml

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (ab6721) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 10 seconds

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 before being incubated with ab1791 overnight at 4°C.

Goat Anti-Rabbit IgG H&L (HRP) (ab6721) secondary antibody was used for detection.

Antibody binding was visualised using ECL development solution **ab133406**.

Histone H3 - ChIP Grade was immunoprecipitated using 0.5mg HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell extract, 5 μ g of Rabbit polyclonal to and 50 μ l of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10 minutes, HeLa whole cell extract lysate diluted in RIPA buffer was added to each sample and incubated for a further 10 minutes under agitation.

Proteins were eluted by addition of 40 μ I SDS loading buffer and incubated for 10 minutes at 70°C; 10 μ I of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab1791.

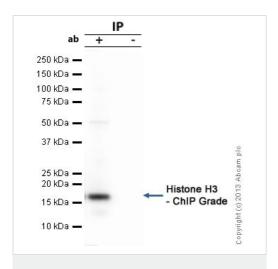
Secondary Antibody: Mouse anti-rabbit HRP light chain (HRP) (ab99697).

Band: 15kDa; Histone H3 - ChIP Grade

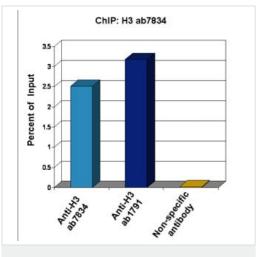
Chromatin from *Xenopus laevis* oocytes was prepared according to the Abcam X-ChIP protocol.

Oocytes were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 mg of chromatin, 3 mg of <u>ab7834</u> (anti-H3, light blue) and 3 μ g of ab1791 (anti-H3, dark blue), and 20 ml of Protein A/G sepharose beads. A non-specific antibody was used as a control (yellow).

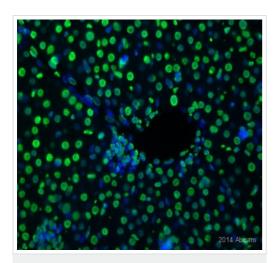
The immunoprecipitated DNA was quantified by real time PCR (Taqman approach).



Immunoprecipitation - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)



ChIP - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

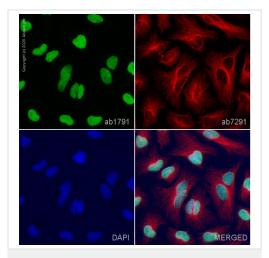


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

This image is courtesy of an anonymous Abreview

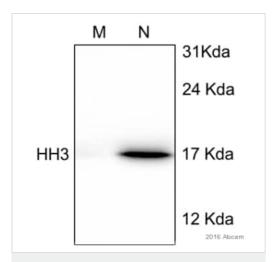
<u>ab1796</u> staining Histone H3 in mouse liver tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections).

Tissue was fixed with paraformaldehyde, permeabilized with 0.05% Triton X-100 in PBS for 30 minutes and blocked with 5% BSA for 1 hour; antigen retrieval was by heat mediation in sodium citrate pH 6. Samples were incubated with the primary antibody (1/500 in blocking buffer) for 16 hours at 4°C. An Alexa Fluor[®] 488-conjugated goat anti-rabbit lgG polyclonal (1/400) was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 antibody - Nuclear Marker and ChIP Grade (ab1791)

ab1791 staining Histone H3 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab1791 at 0.1 µg/mL and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).



Western blot - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

This image is courtesy of an anonymous Abreview

All lanes : Anti-Histone H3 antibody - Nuclear Marker and ChIP Grade (ab1791) at 1/1000 dilution

Lane 1: Mouse skeletal muscle mitochondrial fraction

Lane 2: Mouse skeletal muscle nuclear fraction

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: HRP-conjugated goat anti-rabbit lgG at 1/4000 dilution

Developed using the ECL technique.

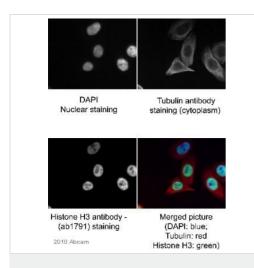
Performed under reducing conditions.

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

Exposure time: 7 minutes

Blocked with 3% milk for 1 hour at 25°C.

Incubated with the primary antibody for 16 hours at 4°C in 3% milk in TBS-tween.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H3 antibody - Nuclear Marker and ChIP Grade (ab1791)

This image is courtesy of an anonymous Abreview

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250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
25 kDa —
20 kDa —
15 kDa —

Western blot - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

ab1791 staining Histone H3 in HeLa (Human epithelial cell line from cervix adenocarcinoma) by ICC/IF

(Immunocytochemistry/immunofluorescence).

Cells were fixed with methanol and blocked with 0.2% fish scale gelatin for 1 hour at 25°C. Samples were incubated with the primary antibody (1/300 in PBS + 0.2% gelatin) for 20 minutes at 25°C. An Alexa Fluor[®] 488-conjugated donkey anti-rabbit lgG polyclonal (1/500) was used as the secondary antibody.

Green - Histone H3.

Blue - DAPI.

Red - Tubulin.

All lanes : Anti-Histone H3 antibody - Nuclear Marker and ChIP Grade (ab1791) at 1 μ g/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2: NIH/3T3 whole cell lysate (ab7179)

Lane 3: Drosophila embryo nuclear extract (from melanogaster embryos 0-12Hr)

Lane 4: S.cerevisiae (Y190) Whole Cell Lysate

Lane 5: S.pombe Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

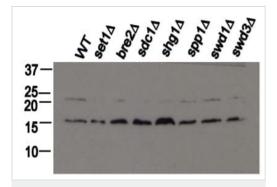
Secondary

 $\begin{tabular}{ll} \textbf{All lanes:} Goat polyclonal to Rabbit lgG - H\&L - Pre-Adsorbed (HRP) at 1/3000 dilution \end{tabular}$

Performed under reducing conditions.

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

ab1791 is tested in western blot on a range of species. We recommend loading higher amounts of protein (20-30ug) to increase the signal in yeast lysates



Western blot - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

This image is courtesy of John E. Mueller and J. Ruth German (Mary Bryk lab)

ChIP: Histone H3 ab1791

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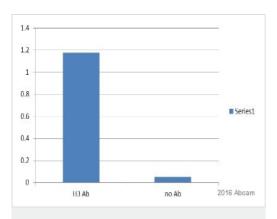
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ChIP - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)



ChIP - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

Rabbit polyclonal to Histone H3 (ab1791) at 1/5000 on S. cerevisiae whole cell lysate (40 ug per lane).

Protein resolved on 15% SDS-PAGE gel. After transfer to PVDF membrane, blots were blocked in 1X PBS, 0.1% Tween-20, and 5% milk. ab1791 was diluted in 5 ml blocking buffer at 1/5000. Blots plus primary antibodies were either incubated overnight at 4°C or at RT for 2 hours. Blots were washed 6X for 10 minutes each in PBS with 0.1% Tween-20 before addition of secondary antibodies. Secondary antibodies were diluted 1/2,000 in blocking buffer and incubated with blots for 2 hours at RT. Secondary blots were washed 4X for 10 minutes each in PBS with 0.1% Tween-20 and 2X for 10 minutes each in PBS.

Chromatin was prepared from HeLa (Human epithelial cell line from cervix adenocarcinoma) cells according to the Abcam X-ChIP protocol.

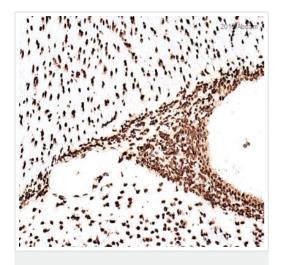
Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25 μ g of chromatin, 2 μ g of ab1791 (blue), and 20 μ l of Protein A/G sepharose beads. No antibody was added to the beads control (yellow).

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.

The ChIP was performed with chromatin from mouse gut cell lysate and ab1791 at 1/250 dilution.

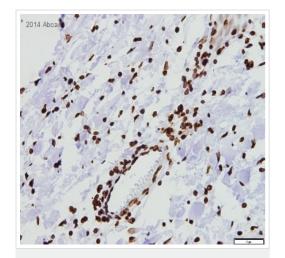
Negative control: No antibody was used (right bar).

The immunoprecipitated DNA was quantified by real time PCR.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

Paraffin-embedded rat brain tissue stained for Histone H3 using ab1791 at 1/8000 dilution in immunohistochemical analysis.

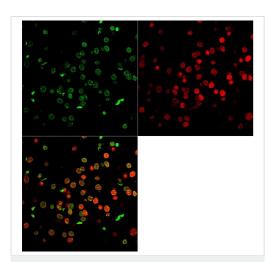


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

This image is courtesy of an anonymous Abreview

ab1791 staining Histone H3 in human infantile fibromatosis tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with formaldehyde and blocked with 1% FBS/BSA for 3 hours at room temperature; antigen retrieval was by heat mediation in Tris pH 9. Samples were incubated with primary antibody (1/100 in TBS + 1% BSA + 1% FBS) for 16 hours. An undiluted HRP-conjugated goat anti-rabbit lgG polyclonal was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Histone H3 antibody - Nuclear Loading Control and ChIP Grade (ab1791)

ab1791 staining Histone H3 (red) in rat brain tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections).

Tissue was fixed with formaldehyde, permeabilized with 0.1% TBS-TritonX and blocked with 10% serum for 1 hour at 25°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with the primary antibody (1/500 in 10% normal goat serum) for 24 hours at 24°C. An Alexa Fluor[®] 594-conjugated goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

Green - Nucleus staining.

Red - Histone H3 staining.

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