

Product datasheet

Anti-Histone H2A antibody - ChIP Grade ab88770

★★★★☆ 2 Abreviews 6 References 8 Images

Overview

Product name	Anti-Histone H2A antibody - ChIP Grade
Description	Rabbit polyclonal to Histone H2A - ChIP Grade
Host species	Rabbit
Tested applications	Suitable for: ChIP, IHC-P, ICC/IF, WB, IP
Species reactivity	<p>Reacts with: Mouse, Cow, Human, Caenorhabditis elegans</p> <p>Predicted to work with: Rat, Chicken, Monkey, Plasmodium falciparum </p>
Immunogen	<p>Synthetic peptide corresponding to Human Histone H2A aa 50 to the C-terminus conjugated to keyhole limpet haemocyanin.</p> <p>(Peptide available as ab100824)</p>
Positive control	<p>This antibody gave a positive signal in the following whole cell lysates: Calf thymus histone; C elegans; HeLa; NIH3T3. IHC-P: FFPE human breast adenocarcinoma tissue sections and human colon.</p>
General notes	<p>Reproducibility is key to advancing scientific discovery and accelerating scientists' next breakthrough.</p> <p>Abcam is leading the way with our range of recombinant antibodies, knockout-validated antibodies and knockout cell lines, all of which support improved reproducibility.</p> <p>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.</p> <p>In preparation for this, we have started to update the applications & species that this product is Abpromise guaranteed for.</p> <p>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.</p> <p>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.</p> <p>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.</p>

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
Clonality	Polyclonal
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab88770** 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		Use 5 µg for 25 µg of chromatin.
IHC-P		Use a concentration of 0.1 - 1 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use a concentration of 1 µg/ml.
WB	★★★★☆	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 14 kDa).
IP		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 H2A family.
Post-translational modifications	The chromatin-associated form is phosphorylated on Thr-121 during mitosis. Deiminated on Arg-4 in granulocytes upon calcium entry. Monoubiquitination of Lys-120 by RING1 and RNF2/RING2 complex gives a specific tag for epigenetic transcriptional repression and participates in X chromosome inactivation of female mammals. It is involved in the initiation of both imprinted and random X inactivation. Ubiquitinated

H2A is enriched in inactive X chromosome chromatin. Ubiquitination of H2A functions downstream of methylation of 'Lys-27' of histone H3. Monoubiquitination of Lys-120 by RNF2/RING2 can also be induced by ultraviolet and may be involved in DNA repair. Following DNA double-strand breaks (DSBs), it is ubiquitinated through 'Lys-63' linkage of ubiquitin moieties by the E2 ligase UBE2N and the E3 ligases RNF8 and RNF168, leading to the recruitment of repair proteins to sites of DNA damage. Monoubiquitination and ionizing radiation-induced 'Lys-63'-linked ubiquitination are distinct events.

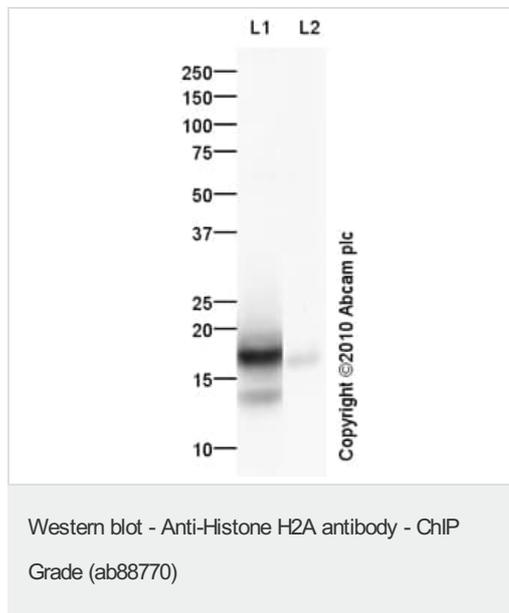
Phosphorylation on Ser-2 is enhanced during mitosis. Phosphorylation on Ser-2 by RPS6KA5/MSK1 directly represses transcription. Acetylation of H3 inhibits Ser-2 phosphorylation by RPS6KA5/MSK1.

Symmetric dimethylation on Arg-4 by the PRDM1/PRMT5 complex may play a crucial role in the germ-cell lineage.

Cellular localization

Nucleus. Chromosome.

Images



All lanes : Anti-Histone H2A antibody - ChIP Grade (ab88770) at 1 µg/ml

Lane 1 : Calf Thymus Histone Preparation Nuclear Lysate

Lane 2 : C.elegans Tissue lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

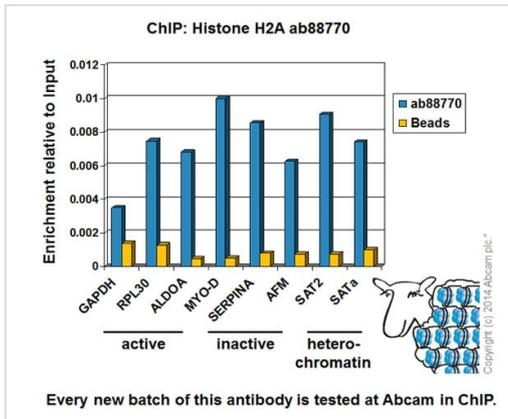
Predicted band size: 14 kDa

Observed band size: 17 kDa

[why is the actual band size different from the predicted?](#)

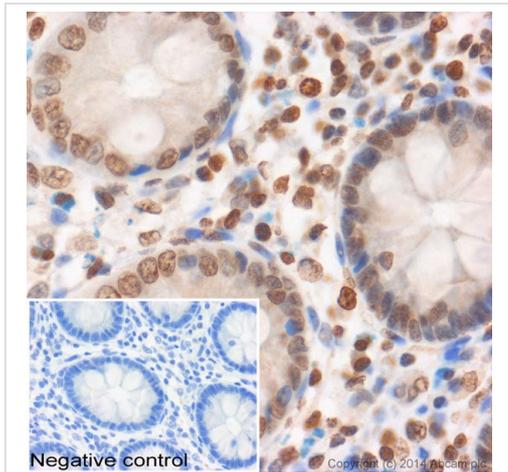
Additional bands at: 14 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 2 minutes



ChIP - Anti-Histone H2A antibody - ChIP Grade (ab88770)

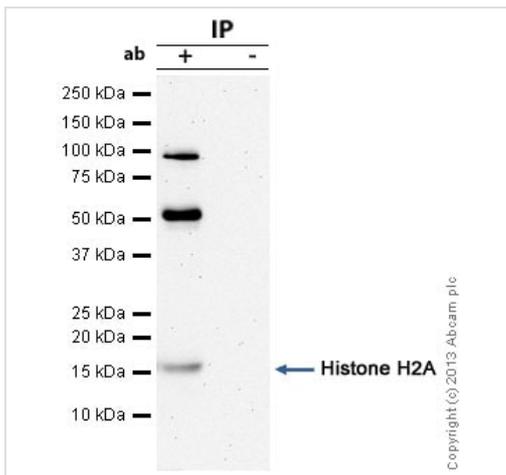
Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 5µg of ab88770 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A antibody - ChIP Grade (ab88770)

IHC image of ab88770 staining Histone H2A in human colon formalin fixed paraffin embedded tissue sections, performed on a Leica Bond. 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 ab88770, 0.1µg/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. No primary antibody was used in the negative control (shown on the inset).

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.



Immunoprecipitation - Anti-Histone H2A antibody - ChIP Grade (ab88770)

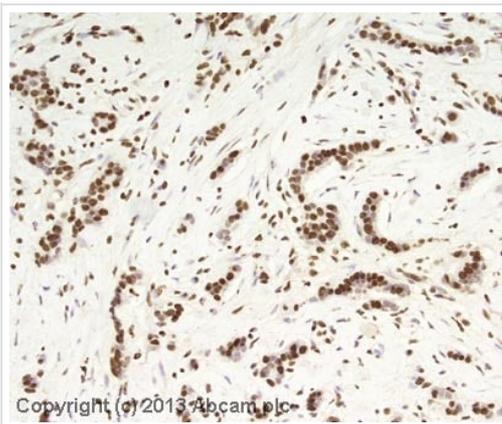
Histone H2A was immunoprecipitated using 0.5mg HeLa whole cell extract, 5µg of Rabbit polyclonal to and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

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

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C; 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab88770.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit IgG light chain (HRP) (ab99697).

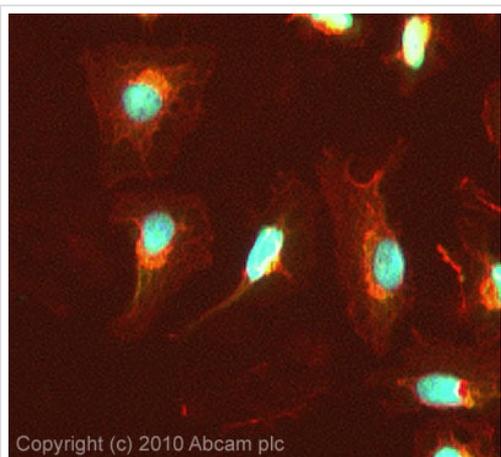
Band: 14kDa, non specific band - 95kDa: We are unsure as to the identity of this extra band; Histone H2A



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A antibody - ChIP Grade (ab88770)

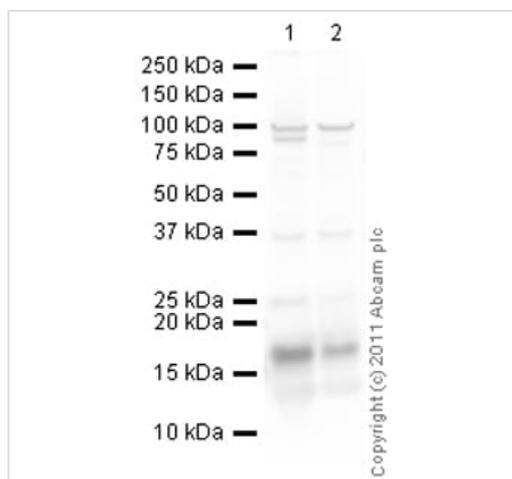
IHC image of Histone H2A staining in human breast adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab88770, 1µg/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.



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Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A antibody - ChIP Grade (ab88770)



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Western blot - Anti-Histone H2A antibody - ChIP Grade (ab88770)

ICC/IF image of ab88770 stained HeLa cells. The cells were 100% methanol fixed (5 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 ab88770 at 1µg/ml overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti- rabbit 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 formaldehyde fixed (4%) (10min) HeLa, Hek293, HepG2, and MCF-7 cells, and in Hek293 and HepG2 Methanol (100%) (5min) fixed cells.

All lanes : Anti-Histone H2A antibody - ChIP Grade (ab88770) at 1 µg/ml

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

Lane 2 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) preadsorbed (ab97080) at 1/5000 dilution

Developed using the ECL technique.

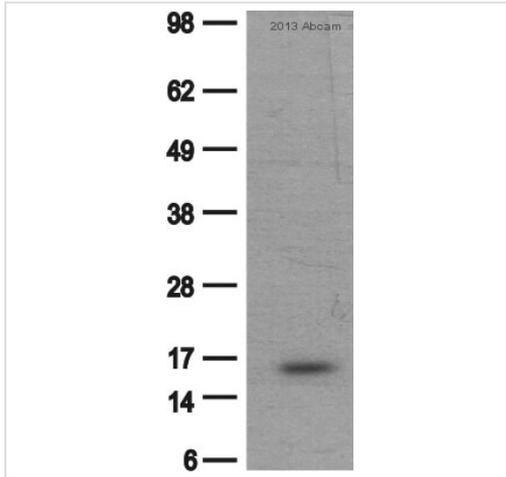
Performed under reducing conditions.

Predicted band size: 14 kDa

Observed band size: 17 kDa [why is the actual band size different from the predicted?](#)

Additional bands at: 100 kDa, 36 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 30 seconds



Western blot - Anti-Histone H2A antibody - ChIP Grade (ab88770)
This image is courtesy of an anonymous Abreview

Anti-Histone H2A antibody - ChIP Grade (ab88770) at 1/1000 dilution + Plasmodium falciparum histone extract at 30000000 cells

Secondary

HRP-conjugated goat anti-rabbit polyclonal IgG at 1/100000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 14 kDa

Observed band size: 16 kDa [why is the actual band size different from the predicted?](#)

Exposure time: 1 minute

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