abcam

Product datasheet

Anti-Histone H2A antibody [EPR17470] - ChIP Grade ab177308

Recombinant RabMAb

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Overview

Product name	Anti-Histone H2A antibody [EPR17470] - ChIP Grade
Description	Rabbit monoclonal [EPR17470] to Histone H2A - ChIP Grade
Host species	Rabbit
Tested applications	**Suitable for:** PepArr, ChIC/CUT&RUN-seq, ChIP, ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa cell lysate and mouse heart, brain, kidney and spleen lysates. IHC-P: Human colon, mouse colon and rat colon tissues. ICC/IF: HeLa cells. ChIP: HeLa cells. ChIC/CUT&RUN-Seq: HeLa cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

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	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR17470

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab177308 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
PepArr		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 2 µg
ChIP		Use 2 µg for 25 µg of chromatin.
ICC/IF		1/500.
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★☆ (2)	1/5000. Detects a band of approximately 14 kDa (predicted molecular weight: 14 kDa).

Target

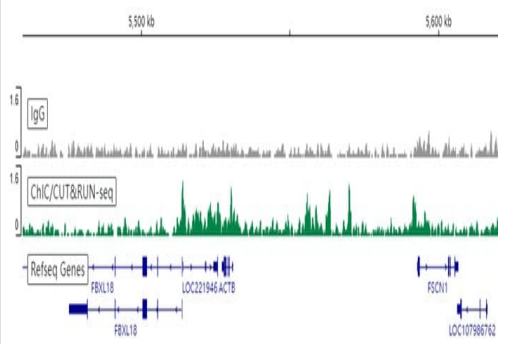
Function Core component of nucleosome. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA replication and chromosomal stability. DNA 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

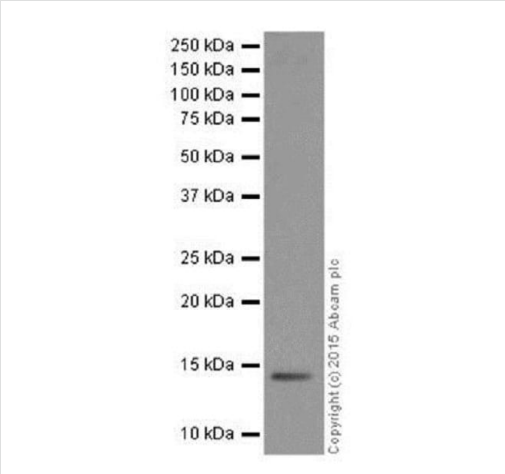


ChIC/CUT&RUN sequencing - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/µL, 2 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 2 µg of ab177308 [EPR17470]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Western blot - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308) at 1/5000 dilution + HeLa (Human epithelial cells from cervix adenocarcinoma) cell lysate at 10 µg

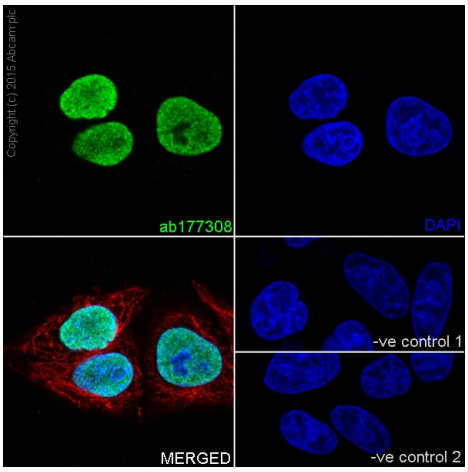
Secondary

Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 14 kDa
Observed band size: 14 kDa

Exposure time: 30 seconds

5% NFDM/TBST: Blocking and diluting buffer.



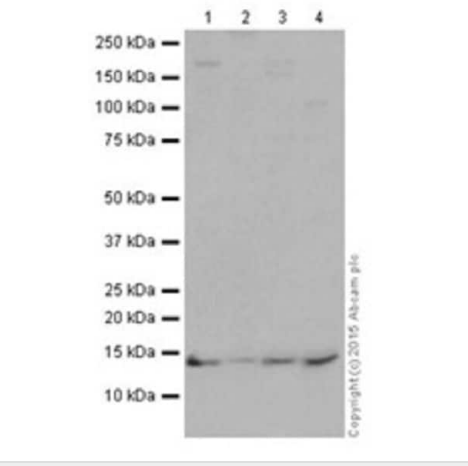
Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H2A with ab177308 at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500 dilution (green).

Confocal image showing nuclear staining on HeLa cell line.

The nuclear counter stain is DAPI (blue).

Tubulin is detected with **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution and **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).
The negative controls are as follows:
1. ab177308 at 1/500 dilution followed by **ab150120** (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution.
2. **ab7291** (anti-Tubulin mouse mAb) at 1/1000 dilution followed by **ab150077** (Alexa Fluor®488 Goat Anti-Rabbit IgG H&L) at 1/500 dilution.



Western blot - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

All lanes : Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308) at 1/1000 dilution

Lane 1 : mouse brain lysate
Lane 2 : mouse heart lysate
Lane 3 : mouse kidney lysate
Lane 4 : mouse spleen lysate

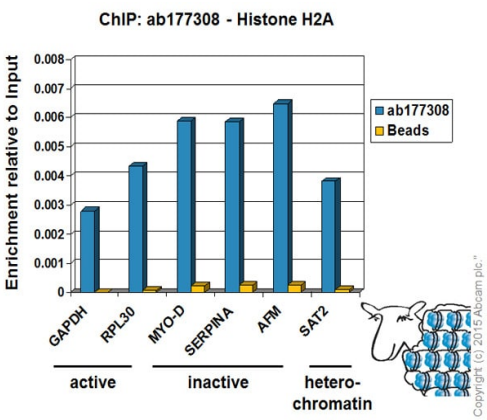
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/50000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 14 kDa
Observed band size: 14 kDa

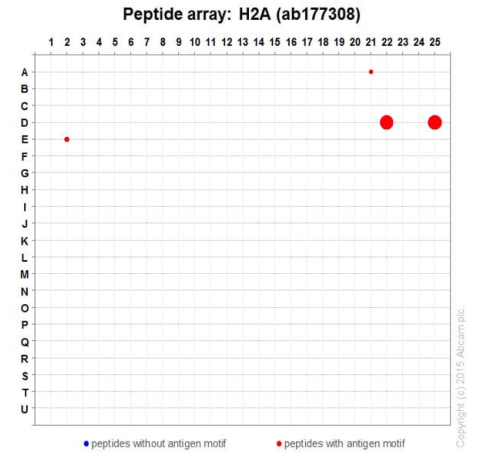
Exposure time: 1 second

5% NFDM/TBST: Blocking and diluting buffer.



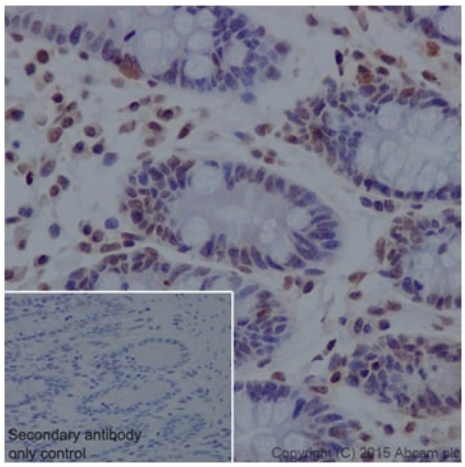
ChIP - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

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, 2µg of ab177308 (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.



Peptide Array - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

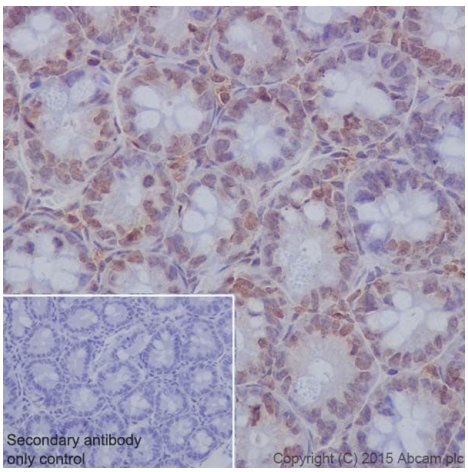
ab177308 was tested in Peptide Array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate).
Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.
The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded **here**.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labeling Histone H2A using ab177308 at 1/1000 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) was used as secondary at 1/500 dilution. Counterstained with Hematoxylin. Nucleus staining on epithelial cells of Human colon tissue was observed.
Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

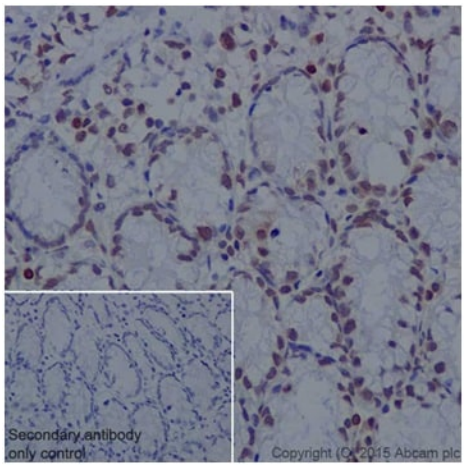
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

Immunohistochemical analysis of paraffin-embedded mouse colon tissue labeling Histone H2A using ab177308 at 1/1000 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) was used as secondary at 1/500 dilution. Counterstained with Hematoxylin. Nucleus staining on epithelial cells of mouse colon tissue was observed.
Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution..

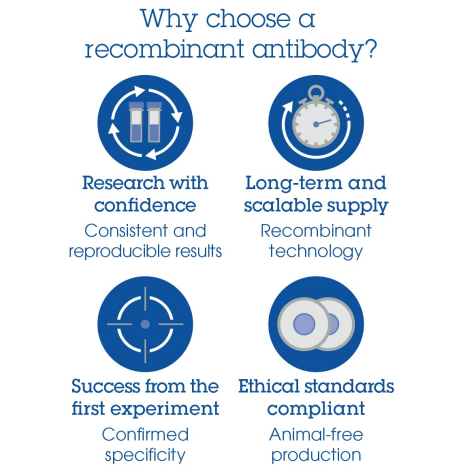
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

Immunohistochemical analysis of paraffin-embedded rat colon tissue labeling Histone H2A using ab177308 at 1/1000 dilution. A Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) was used as secondary at 1/500 dilution. Counterstained with Hematoxylin. Nucleus staining on epithelial cells of rat colon tissue was observed. Negative control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Anti-Histone H2A antibody [EPR17470] - ChIP Grade (ab177308)

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