abcam

Product datasheet

# Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free ab217840

Recombinant RabMAb

1 Abreviews 7 Images

## Overview

| | |
|---|---|
| **Product name** | Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free |
| **Description** | Rabbit monoclonal [EPR17470] to Histone H2A - ChIP Grade – BSA and Azide free |
| **Host species** | Rabbit |
| **Tested applications** | **Suitable for:** WB, PepArr, IHC-P, ChIP, ICC/IF, ChIC/CUT&RUN-seq |
| **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** | ab217840 is the carrier-free version of **ab177308**.<br><br>Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.<br><br>This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.<br><br>Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.<br><br>This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.<br><br>This product is a recombinant monoclonal antibody, which offers several advantages including:<br>- High batch-to-batch consistency and reproducibility<br>- Improved sensitivity and specificity<br>- Long-term security of supply<br>- Animal-free production<br>For more information **see here**.<br><br>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. Do Not Freeze. |
| **Storage buffer** | pH: 7.2<br>Constituent: PBS |
| **Carrier free** | Yes |
| **Purity** | Protein A purified |
| **Clonality** | Monoclonal |
| **Clone number** | EPR17470 |
| **Isotype** | IgG |

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab217840 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** | | Use at an assay dependent concentration. Detects a band of approximately 14 kDa (predicted molecular weight: 14 kDa). |
| **PepArr** | | Use at an assay dependent concentration. |
| **IHC-P** | | Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| **ChIP** | | Use 2 µg for 25 µg of chromatin. |
| **ICC/IF** | | Use at an assay dependent concentration. |
| **ChIC/CUT&RUN-seq** | | Use at an assay dependent concentration. |

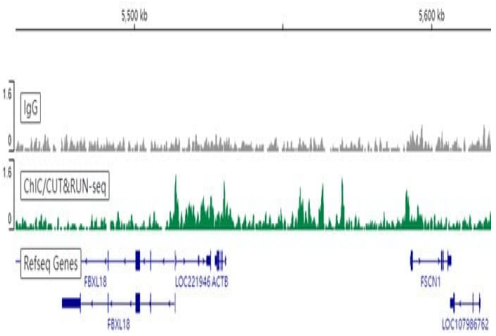
## Target

| | |
|---|---|
| **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.<br>Deiminated on Arg-4 in granulocytes upon calcium entry.<br>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



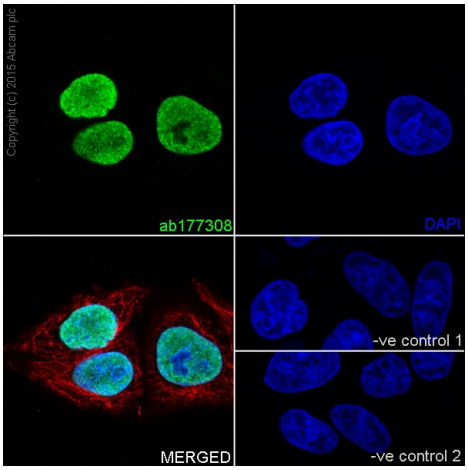

ChIC/CUT&RUN sequencing - Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free (ab217840)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab177308**).




Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free (ab217840)

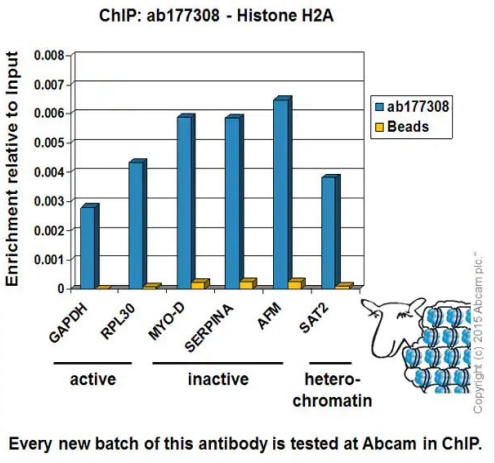
Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cells from cervix adenocarcinoma) cells labeling Histone H2A with **ab177308** at 1/500 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/500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

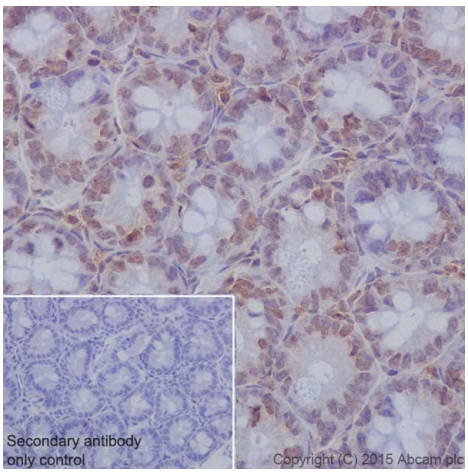
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab177308**).




ChIP - Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free (ab217840)

This data was developed using the same antibody clone in a different buffer formulation (**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.

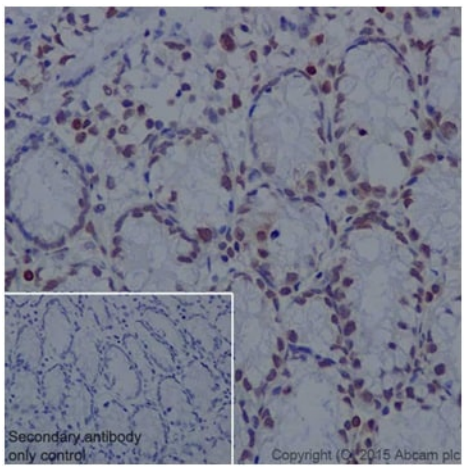



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free (ab217840)

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..

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab177308**).

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

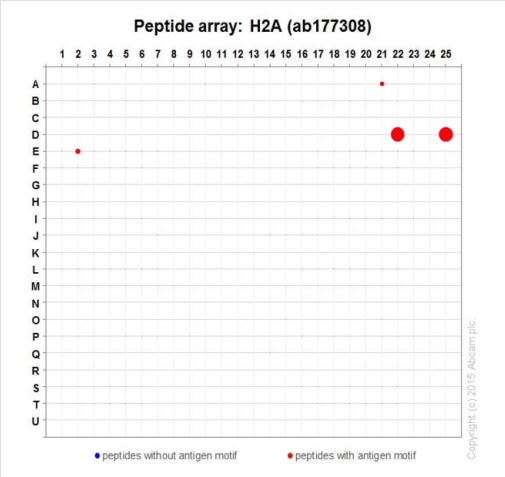



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free (ab217840)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab177308**).

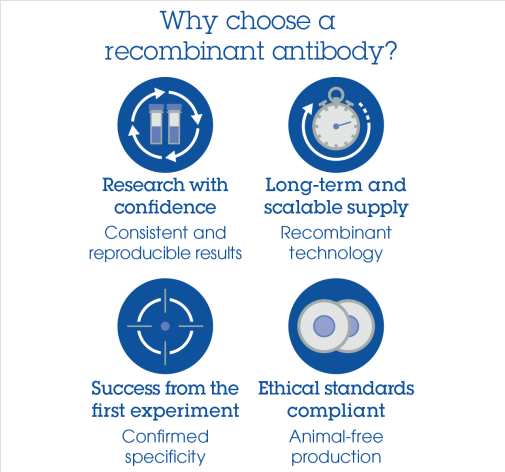
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.




Peptide Array - Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free (ab217840)

**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**.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab177308**).




Anti-Histone H2A antibody [EPR17470] - ChIP Grade - BSA and Azide free (ab217840)

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