abcam

Product datasheet

Anti-Histone H2A antibody - ChIP Grade ab18255

★★★★★ 21 Abreviews 125 References 9 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:** ICC/IF, WB, IP, ChIP
Species reactivity	**Reacts with:** Mouse, Cow, Human
Immunogen	Synthetic peptide within Human Histone H2A aa 100 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. The exact sequence is proprietary. (Peptide available as **ab19751**)
Positive control	WB: HeLa nuclear extract. Calf thymus histone preparation. Histone H2A Recombinant Protein. ChIP: Chromatin from U-2 OS cells. IP: Histone H2A IP in HeLa whole cell lysate. ICC/IF: HeLa cells.
General notes	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 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

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab18255 in the following tested applications.

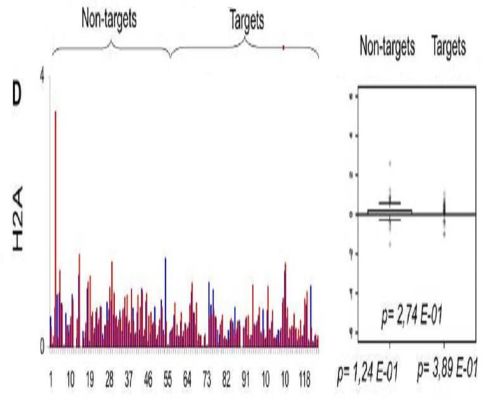
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★★ (4)	1/200. see Abreview submitted by Kirk McManus
WB	★★★★★ (13)	Use a concentration of 1 µg/ml. Detects a band of approximately 14 kDa (predicted molecular weight: 14 kDa).
IP		Use a concentration of 5 µg/ml.
ChIP	★★★★★ (2)	Use at an assay dependent concentration. Every new batch of this antibody is tested at Abcam in ChIP

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.

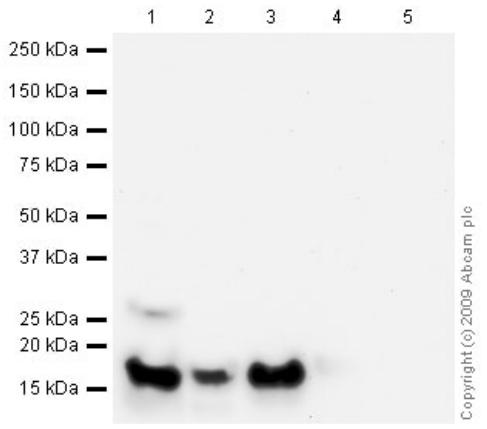
Images



ChIP - Anti-Histone H2A antibody - ChIP Grade (ab18255)

Myc affects the incorporation levels of histone variant H2A.Z.

qChIP was performed using specific antibodies recognizing A. H2A.Z, B. H2A.Zac, **Panel D. H2A**, E. H2AK5ac and G. H1. All the qChIP values are expressed as % of input and normalized for total histone H3, with the exception of C and F, where H2A.Z acetylation is noramlized for H2A.Z density, and H2AK5 acetylation is normalized for H2A density, respectively. The box plots show the fold change distribution of each acetylated residue for the two subpopulations.



Western blot - Anti-Histone H2A antibody - ChIP Grade (ab18255)

All lanes : Anti-Histone H2A antibody - ChIP Grade (ab18255) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate at 10 µg
Lane 2 : Calf Thymus Histone Preparation Nuclear Lysate at 0.5 µg
Lane 3 : Histone H2A Recombinant Protein at 0.1 µg
Lane 4 : Histone H3.1 Recombinant Protein at 0.1 µg
Lane 5 : Histone H4 Recombinant Protein at 0.1 µg

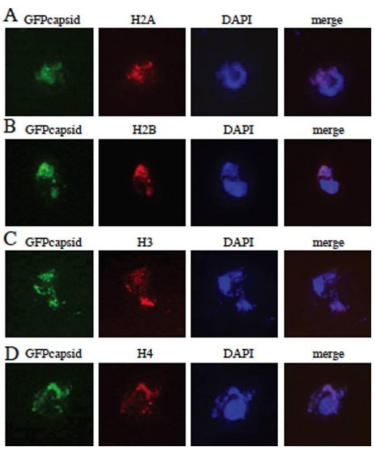
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 14 kDa
Observed band size: 17 kDa

Exposure time: 3 minutes

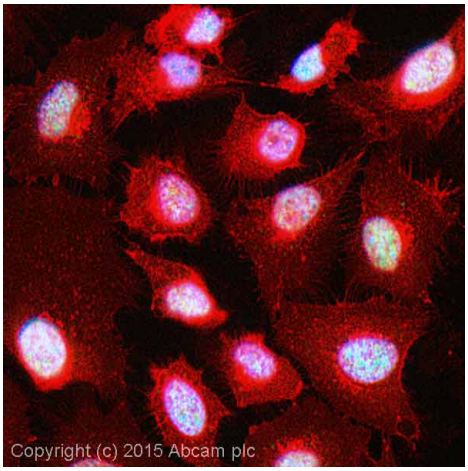


Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A antibody - ChIP Grade (ab18255)

Colpitts et al PLoS One. 2011;6(9):e24365. doi: 10.1371/journal.pone.0024365. Epub 2011 Sep 1. Fig 2.

DENV C colocalizes with histones in Huh7 liver cells.

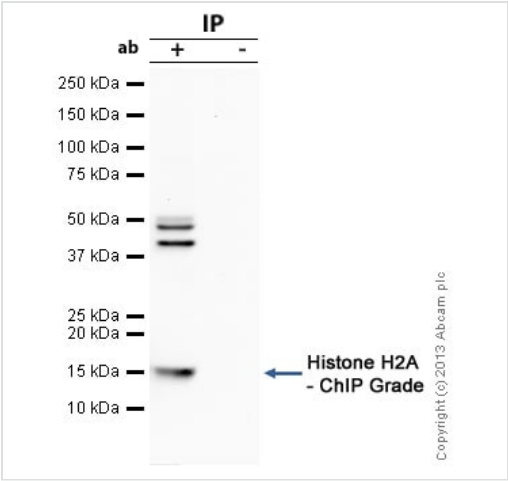
DEN2 C colocalized with **H2A (A),** H2B (B), H3 (C) and H4 (D) in Huh7 cells. Cells were transfected with GFP-DEN2 C and fixed in 4% paraformaldehyde 48 h post-transfection. Cells were stained with antibodies against histones and a TRITC secondary antibody. Cells were counterstained with DAPI to visualize the nucleus. GFP-DEN2 C expression is green, histone staining is red and DAPI is blue.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A antibody - ChIP Grade (ab18255)

ICC/IF image of ab18255 stained HeLa (Human epithelial cell line from cervix adenocarcinoma) cells.

The cells were fixed in 100% methanol (5 min) then permeabilized using 0.1% PBS-Triton and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to further permeabilize the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab18255 at 1 µg/ml overnight at +4°C. The secondary antibody (pseudo-colored green) was Alexa Fluor® 488 goat anti- rabbit (**ab150081**) IgG (H+L) preadsorbed, used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1h at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43 µM for 1 hour at room temperature.



Immunoprecipitation - Anti-Histone H2A antibody - ChIP Grade (ab18255)

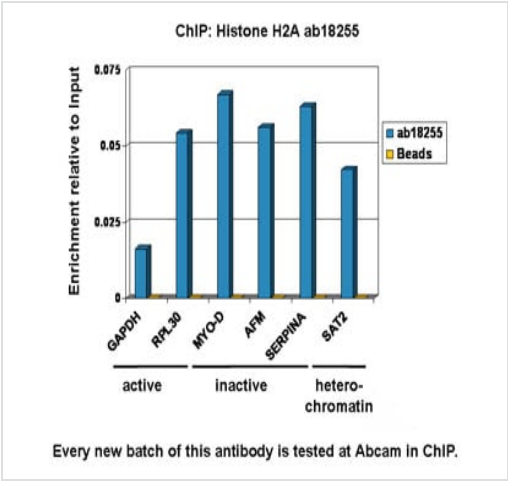
Histone H2A - ChIP Grade was immunoprecipitated using 0.5 mg HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell extract, 5 µg of Rabbit polyclonal to and 50 µL of protein G magnetic beads (+). No antibody was added to the control (-).

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

Proteins were eluted by addition of 40 µL SDS loading buffer and incubated for 10 min at 70°C; 10 µL of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab18255.

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

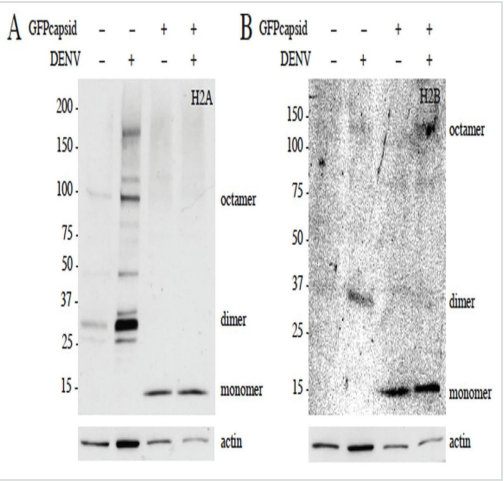
Band: 14kDa, non specific bands - 42kDa: We are unsure as to the identity of this extra band; Histone H2A - ChIP Grade



ChIP - Anti-Histone H2A antibody - ChIP Grade (ab18255)

Chromatin was prepared from U-2 OS (Human bone osteosarcoma epithelial cell line) 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, 6 µL of ab18255 (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.

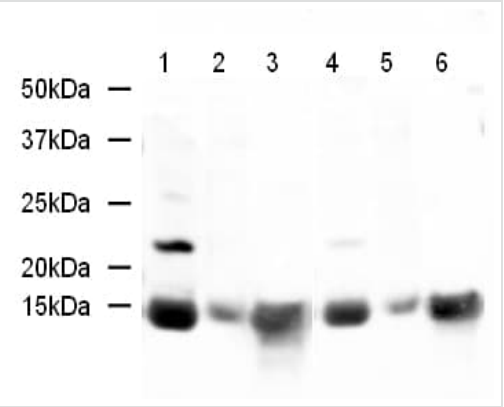


Western blot - Anti-Histone H2A antibody - ChIP Grade (ab18255)

DENV expression disrupts histone oligomerization.

Panel A and B shown:

Huh7 cells were transfected with DEN2 C and/or infected with DEN2 24 h post-transfection. Cells were lysed 24 h post-infection (48 h post-transfection) and lysates were run on 4–12% SDS-PAGE gel. Gels were used in a Western blotting assay with antibodies against histones H2A ab18255 (A), H2B (B), H3 (C) and H4 (D); monomers, dimers and octamers are indicated. Gels were stripped and reprobed with an antibody against actin as a protein loading control. The same amount of protein was loaded in each lane for each gel as a control for expression.



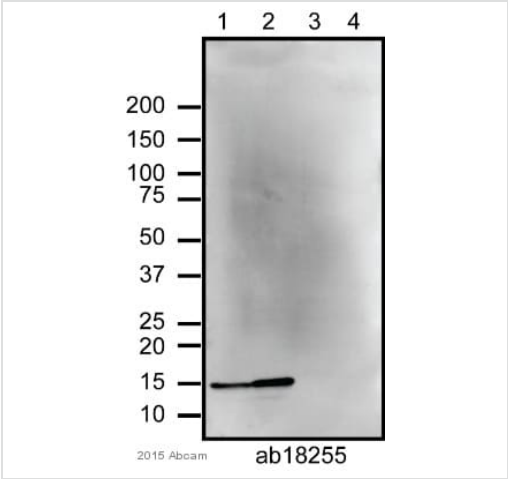
Western blot - Anti-Histone H2A antibody - ChIP Grade (ab18255)

All lanes : Anti-Histone H2A antibody - ChIP Grade (ab18255) at 1 µg/ml

Lane 1 : HeLa (Human epithelial cell line from cervix adenocarcinoma) lysate at 20 µg
Lane 2 : HeLa nuclear lysate at 20 µg
Lane 3 : Calf thymus histone lysate at 20 µg
Lane 4 : HeLa lysate at 1 µg/ml with Human Histone H2A peptide (**ab19751**) at 1 µg/ml
Lane 5 : HeLa nuclear lysate at 1 µg/ml with Human Histone H2A peptide (**ab19751**) at 1 µg/ml
Lane 6 : Calf thymus histone lysate at 1 µg/ml with Human Histone H2A peptide (**ab19751**) at 1 µg/ml

Predicted band size: 14 kDa
Observed band size: 14 kDa
Additional bands at: 22 kDa (possible cross reactivity)

ab18255 is partially blocked by the immunizing peptide **ab19751**. There is an additional band at 22kDa in HeLa lysate which is attributed to cross-reactivity.



Western blot - Anti-Histone H2A antibody - ChIP Grade (ab18255)

This image is courtesy of an Abreview submitted by Ragnhild Eskeland

All lanes : Anti-Histone H2A antibody - ChIP Grade (ab18255) at 1/1000 dilution

Lane 1 : Native recombinant octamers K562 cells
Lane 2 : Recombinant Human octamers containing H2A
Lane 3 : Recombinant Human octamers containing H2A.Z.2.1
Lane 4 : Recombinant Human octamers containing H2A.Z.1

Lysates/proteins at 0.5 µg per lane.

Secondary
All lanes : HRP-conjugated donkey anti-rabbit IgG polyclonal at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 14 kDa
Observed band size: 15 kDa

Exposure time: 5 minutes

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