abcam

Product datasheet

Anti-Histone H2A antibody - ChIP Grade ab15653

8 References 4 Images

Overview

Product name	Anti-Histone H2A antibody - ChIP Grade
Description	Rabbit polyclonal to Histone H2A - ChIP Grade
Host species	Rabbit
Specificity	ab15653 was generated using an immunogen specific to the C-terminal of H2A2B (SwissProt Q8IUE6). Interestingly mass spec analysis on immunoprecipitated material using ab15653 indicates that the antibody recognises all isotypes of H2A.
Tested applications	**Suitable for:** WB, ICC/IF, ChIP
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide corresponding to Human Histone H2A aa 100 to the C-terminus (C terminal) conjugated to keyhole limpet haemocyanin. (Peptide available as ab15660)
Positive control	WB: HeLa nuclear extract, NIH3T3 cells; ICC: HeLa
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 ab15653 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 a concentration of 0.5 µg/ml. Detects a band of approximately 18 kDa (predicted molecular weight: 14 kDa).
ICC/IF		Use a concentration of 1 µg/ml.
ChIP		Use 2-25 µg for µg of chromatin.

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

Western blot - Anti-Histone H2A antibody - ChIP Grade (ab15653)

All lanes : Anti-Histone H2A antibody - ChIP Grade (ab15653) at 1 µg/ml

Lane 1 : CTH cell lysate at 0.5 µg
Lane 2 : HeLa whole cell lysate at 20 µg
Lane 3 : HeLa Nuclear Triton Enriched cell lysate at 20 µg
Lane 4 : NIH/3T3 whole cell lysate at 20 µg
Lane 5 : NIH/3T3 Nuclear Triton Enriched cell lysate at 20 µg
Lane 6 : Histone H3 Recombinant Protein (Negative control) at 0.1 µg

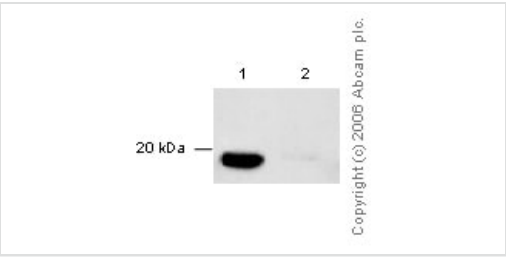
Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

Predicted band size: 14 kDa
Additional bands at: 17 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 30 seconds

Blocking buffer: 3% Milk

Gel type: MES



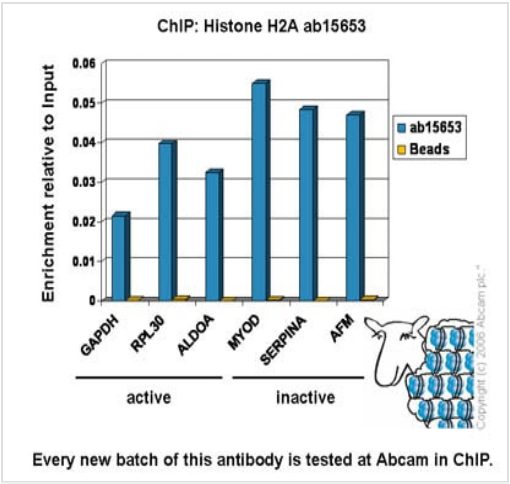
Western blot - Anti-Histone H2A antibody - ChIP Grade (ab15653)

All lanes : Anti-Histone H2A antibody - ChIP Grade (ab15653) at 0.5 µg/ml

Lane 1 : HeLa nuclear extract
Lane 2 : HeLa nuclear extract with Human Histone H2A peptide (ab15660) at 1 µg/ml

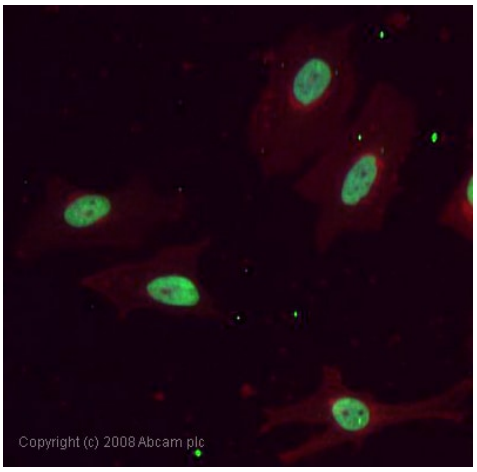
Predicted band size: 14 kDa
Observed band size: 18 kDa

ab15653 recognises H2A in HeLa nuclear extracts (lane1), which is successfully blocked using the immunizing peptide (lane2).



ChIP - Anti-Histone H2A antibody - ChIP Grade (ab15653)

Chromatin was prepared from Hela cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10min. The ChIP was performed with 25µg of chromatin, 2µg of ab15653 (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). Primers and probes are located in the first kb of the transcribed region.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A antibody - ChIP Grade (ab15653)

ICC/IF image of ab15653 stained human HeLa cells. The cells were 4% PFA fixed (10 min), permabilised in 0.1% PBS-Tween (20 min) and incubated with the antibody (ab15653, 1µg/ml) for 1h at room temperature. 1%BSA / 10% normal goat serum / 0.3M glycine was used to block non-specific protein-protein interactions. 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). DAPI was used to stain the cell nuclei (blue).

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