abcam

Product datasheet

Anti-Histone H2A (symmetric di methyl R3) antibody ab22397

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Overview

Product name	Anti-Histone H2A (symmetric di methyl R3) antibody
Description	Rabbit polyclonal to Histone H2A (symmetric di methyl R3)
Host species	Rabbit
Tested applications	**Suitable for:** IHC-P, WB, ICC/IF, ELISA
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide corresponding to Human Histone H2A aa 1-100 conjugated to keyhole limpet haemocyanin. (Peptide available as ab22399)
Positive control	IHC-P: Human normal kidney FFPE tissue sections. ICC/IF: MCF-7 cells WB: Calf Thymus Histone, HeLa lysate, and Histone H2A Recombinant.
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 ab22397 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
IHC-P		Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 14 kDa).
ICC/IF		Use a concentration of 5 µg/ml.
ELISA		Use at an assay dependent concentration. This antibody gave a positive result in ELISA against the immunizing peptide (ab22399). It gave a negative result in ELISA against the non-modified equivalent peptide (ab13186). This indicates that it is specific for the modified peptide. See figure below. Not yet tested in other applications. Optimal dilutions/concentrations should be determined by the end user.

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.

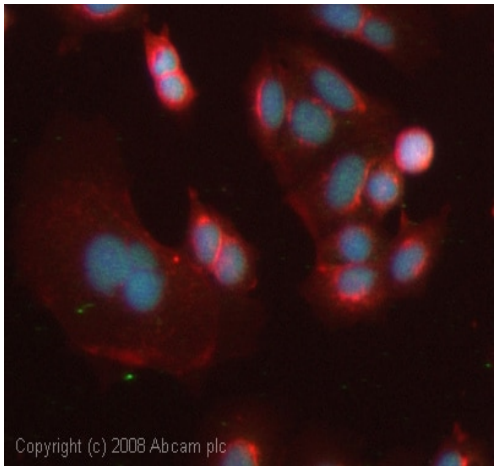
Sequence similarities Belongs to the histone H2A family.

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.

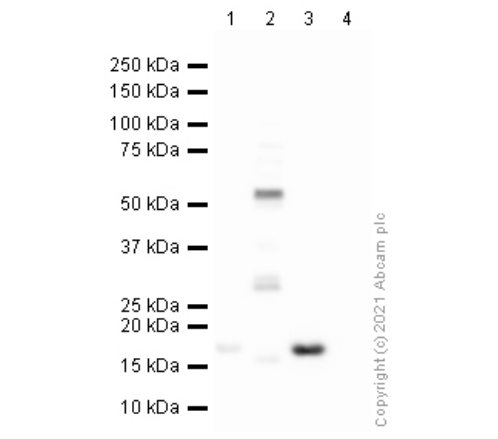
Cellular localization Nucleus. Chromosome.

Images



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A (symmetric di methyl R3) antibody (ab22397)

ICC/IF image of ab22397 stained human MCF7 cells. The cells were methanol fixed (5 min), permabilised in 0.1% PBS-Tween (20 min) and incubated with the antibody (ab22397, 5µg/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). This antibody also gave a positive IF result in HeLa, HEK 293 and HepG2 cells.



Western blot - Anti-Histone H2A (symmetric di methyl R3) antibody (ab22397)

All lanes : Anti-Histone H2A (symmetric di methyl R3) antibody (ab22397) at 1 µg/ml

Lane 1 : Calf Thymus Histone at 0.5 µg
Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg
Lane 3 : Histone H2A Recombinant at 0.1 µg
Lane 4 : Histone H3.1 Recombinant (negative control) at 0.1 µg

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

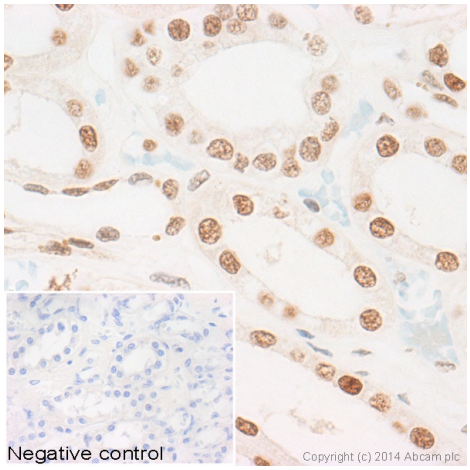
Developed using the ECL technique.

Performed under reducing conditions.

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

Exposure time: 2 minutes

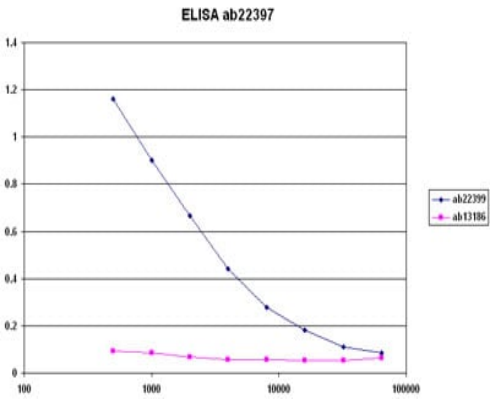
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% Milk before being incubated with ab22397 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A (symmetric di methyl R3) antibody (ab22397)

IHC image of ab22397 staining Histone H2A (symmetric di methyl R3) in human kidney 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 ab22397, 5µg/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.



ELISA - Anti-Histone H2A (symmetric di methyl R3) antibody (ab22397)

This antibody gave a positive result in ELISA against the immunizing peptide ab22399 (blue line). Histone H2A (unmodified) peptide ab13186 (pink line) gave a negative result, This indicates that it is specific for the Histone H2A (symmetric di methyl R3) peptide.

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