abcam

Product datasheet

Anti-mH2A1 antibody ab37264

KO VALIDATED

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Overview

Product name	Anti-mH2A1 antibody
Description	Rabbit polyclonal to mH2A1
Host species	Rabbit
Specificity	ab37264 recognises the three known isoforms of mH2A1 including mH2A1.2 (longest isoform) and the mH2A1.1 (shortest isoform). We have had varying reports about the efficiency with which this antibody recognises mH2A1 in mouse cells and tissues. Please contact our Scientific Support team if you have any queries about this.
Tested applications	**Suitable for:** ChIP, WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat, Chicken, Cow, Xenopus laevis
Immunogen	Synthetic peptide corresponding to Human mH2A1 aa 150-250 conjugated to keyhole limpet haemocyanin. (Peptide available as **ab37263**)
Positive control	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 ab37264 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
ChIP		Use at an assay dependent concentration. PubMed: 19380460
WB	★★★☆☆ (1)	Use a concentration of 1 µg/ml. Detects a band of approximately 40 kDa (predicted molecular weight: 40 kDa).
IHC-P	★★★★★ (1)	Use a concentration of 5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
ICC/IF	★★★★☆ (1)	Use a concentration of 1 - 5 µg/ml.

Target

Function Variant histone H2A which replaces conventional H2A in a subset of nucleosomes where it represses transcription. 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. Involved in stable X chromosome inactivation. Inhibits the binding of transcription factors and interferes with the activity of remodeling SWI/SNF complexes. Inhibits histone acetylation by EP300 and recruits class I HDACs, which induces an hypoacetylated state of chromatin. In addition, isoform 1, but not isoform 2, binds ADP-ribose and O-acetyl-ADP-ribose, and may be involved in ADP-ribose-mediated chromatin modulation.

Tissue specificity Ubiquitous.

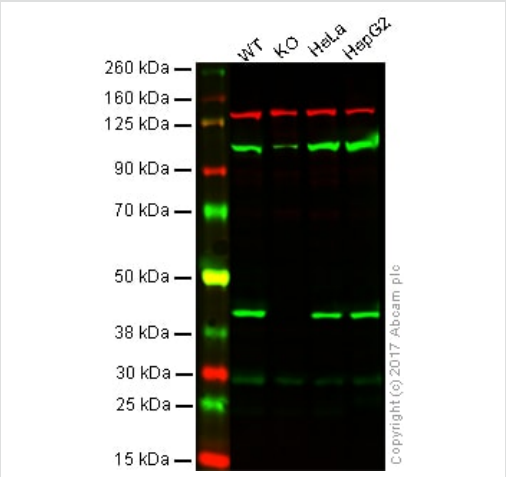
Sequence similarities Contains 1 histone H2A domain.
Contains 1 Macro domain.

Post-translational modifications Monoubiquitinated at either Lys-116 or Lys-117. May also be polyubiquitinated. Ubiquitination is mediated by the CUL3/SPOP E3 complex and does not promote proteasomal degradation. Instead, it is required for enrichment in inactive X chromosome chromatin.

Cellular localization Nucleus. Chromosome. Enriched in inactive X chromosome chromatin and in senescence-

associated heterochromatin.

Images

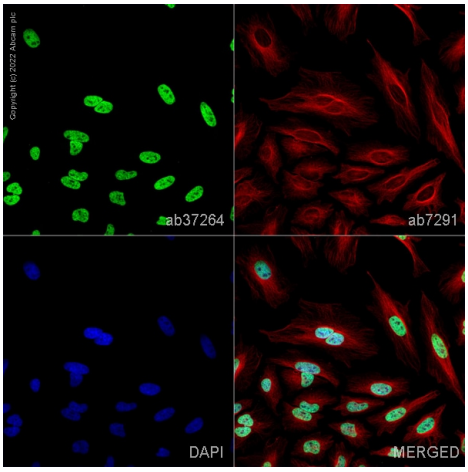


Western blot - Anti-mH2A1 antibody (ab37264)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: MH2A1 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: HepG2 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab37264 observed at 40 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab37264 was shown to specifically recognize MH2A1 in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when MH2A1 knockout cells were examined. Wild-type and MH2A1 knockout samples were subjected to SDS-PAGE. Ab37264 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 1 ug/ml and 1/10,000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

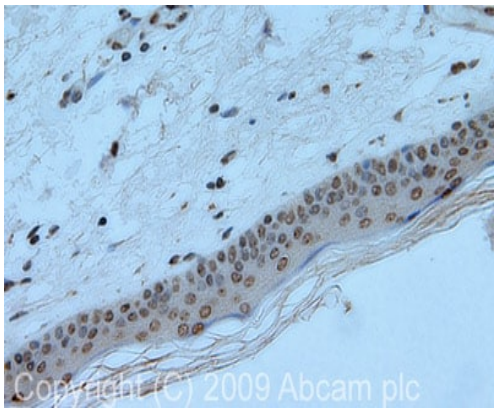


Immunocytochemistry/ Immunofluorescence - Anti-mH2A1 antibody (ab37264)

ab37264 staining mH2A1 in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab37264 at 1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

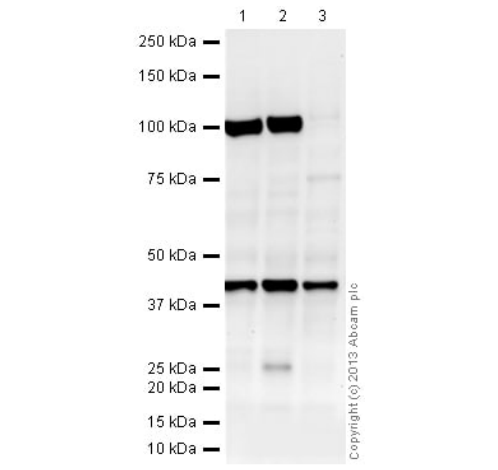
Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mH2A1 antibody (ab37264)

IHC image of mH2A1 staining in human skin FFPE section, performed on a Leica Bond™ system using the standard protocol F. 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 ab37264, 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.



Western blot - Anti-mH2A1 antibody (ab37264)

All lanes : Anti-mH2A1 antibody (ab37264) at 1 µg

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate
Lane 2 : HepG2 (Human hepatocellular liver carcinoma cell line) Whole Cell Lysate
Lane 3 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 40 kDa
Observed band size: 40 kDa
Additional bands at: 100 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 16 minutes

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