abcam

Product datasheet

Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free ab232602

KO VALIDATED Recombinant RabMAb

1 References 8 Images

Overview

Product name	Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free
Description	Rabbit monoclonal [EPR9359(2)] to mH2A1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HepG2, HEK293T and HeLa whole cell lysates. ICC/IF: MCF7 and HeLa cells. IHC-P: Human liver and kidney tissues.
General notes	ab232602 is the carrier-free version of **ab183041**. 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. 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. 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. 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. This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. 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 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR9359(2)
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab232602 in the following tested applications.

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

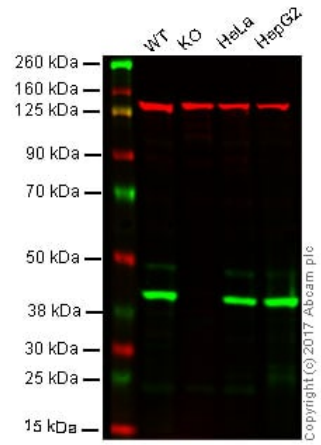
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 40 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.

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

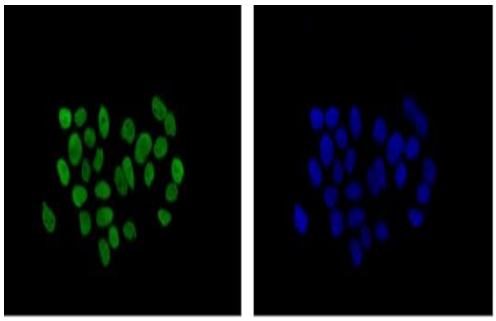


Western blot - Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free (ab232602)

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 - **ab183041** observed at 40 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab183041 was shown to specifically react with mH2A1 when mH2A1 knockout samples were used. Wild-type and mH2A1 knockout samples were subjected to SDS-PAGE. Ab183041 and **ab18058** (Mouse anti Vinculin loading control) were incubated overnight at 4°C at 10000 dilution and 1/10000 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 1/10000 dilution for 1 hour at room temperature before imaging.

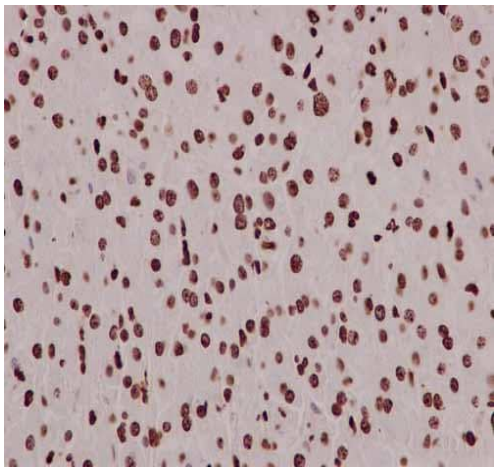
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183041**).



Immunocytochemistry/ Immunofluorescence - Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free (ab232602)

Immunofluorescent analysis of 4% paraformaldehyde-fixed MCF7 cells labeling mH2A1 with **ab183041** at 1/250 dilution followed by Goat anti rabbit IgG (Alexa Fluor® 488) at 1/200 dilution (green). Counter stained with Dapi (blue).

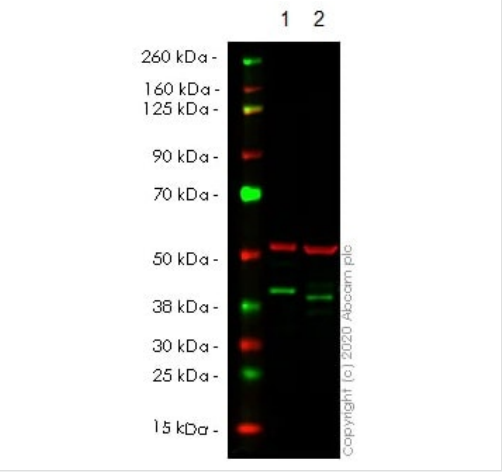
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183041**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free (ab232602)

Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling mH2A1 with **ab183041** at 1/100 dilution followed by prediluted HRP Polymer for Rabbit IgG. Counter stained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183041**).



Western blot - Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free (ab232602)

All lanes : Anti-mH2A1 antibody [EPR9359(2)] (**ab183041**) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : H2AFY CRISPR/Cas9 edited HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

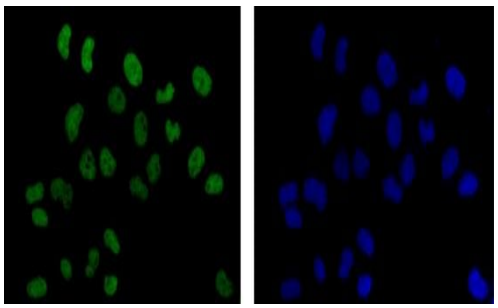
Predicted band size: 40 kDa
Observed band size: 40 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab183041**).

Lanes 1- 2: Merged signal (red and green). Green - **ab183041** observed at 40 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab183041 was shown to react with mH2A1 in wild-type HEK-293T cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab266241** (CRISPR/Cas9 edited cell lysate **ab257463**) lane below 40kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HEK-293T and H2AFY CRISPR/Cas9 edited HEK-293T cell lysates were subjected to SDS-PAGE. Membrane was blocked for

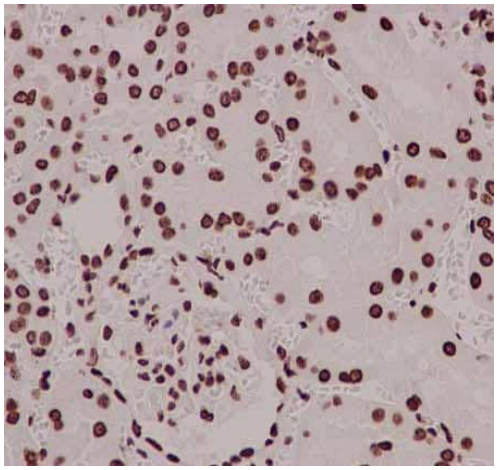
1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab183041** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free (ab232602)

Immunofluorescent analysis of acetone-fixed HeLa cells labeling mH2A1 with **ab183041** at 1/250 dilution followed by Goat anti rabbit IgG (Alexa Fluor® 488) at 1/200 dilution (green). Counter stained with Dapi (blue).

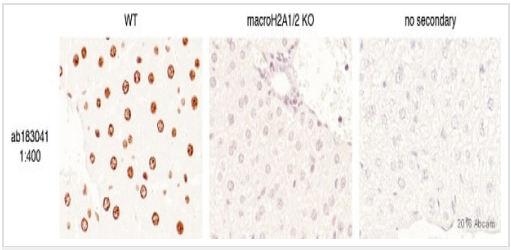
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183041**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free (ab232602)

Immunohistochemical analysis of paraffin-embedded Human kidney tissue labeling mH2A1 with **ab183041** at 1/100 dilution followed by prediluted HRP Polymer for Rabbit IgG. Counter stained with Hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183041**).

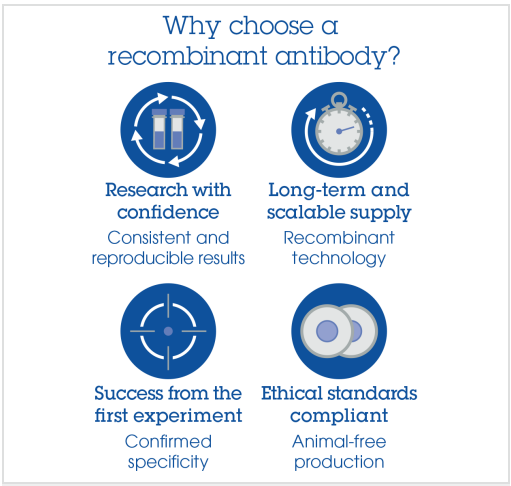


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-mH2A1 antibody [EPR9359(2)] - BSA and Azide free (ab232602)

This image is courtesy of an anonymous abreview.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse liver from wild-type and mH2A1/2 knock out tissue sections labeling mH2A1 with **ab183041** at 1/400 dilution. Sections were fixed in formaldehyde; heat mediated antigen retivial was performed using a citrate buffer pH 6. An undiluted polyclonal horse anti-rabbit IgG (HRP-conjugated) was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab183041**).



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