abcam

Product datasheet

Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker ab124781

Recombinant RabMAb

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Overview

Product name	Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker
Description	Rabbit monoclonal [EPR895] to Histone H2A.X - Nuclear Marker
Host species	Rabbit
Specificity	This antibody does not work in ICC/IF on PFA fixed cells but does work on methanol fixed cells. The immunogen used for this product shares full homology with other H2A proteins, including H2A1, H2A1B, H2A2A and H2AZ. Cross-reactivity with these proteins has not been confirmed experimentally.
Tested applications	**Suitable for:** WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Raji and HEK293 cell lysates, fetal kidney, human heart, mouse and rat kidney tissue lysates. IHC-P: Human kidney and clear cell carcinoma tissues. ICC/IF: HeLa cells. IP: HeLa whole cell lysate (**ab150035**).
General notes	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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Stable for 12 months at -20°C.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide

	Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR895
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab124781 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	★★★★★ (3)	1/1000 - 1/10000. Detects a band of approximately 14 kDa (predicted molecular weight: 15 kDa).
IP		Use a concentration of 5 µg/ml.
IHC-P		1/500 - 1/1000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★★ (1)	1/1000. ab124781 does not work on PFA fixed cells but does work on methanol fixed cells.
Flow Cyt (Intra)		Use at an assay dependent concentration.

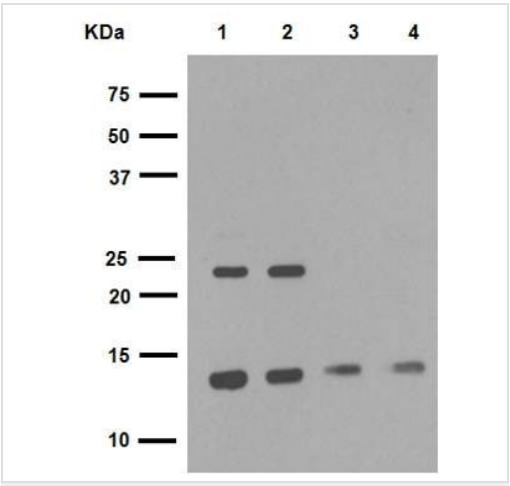
Target

Function	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. 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. Required for checkpoint-mediated arrest of cell cycle progression in response to low doses of ionizing radiation and for efficient repair of DNA double strand breaks (DSBs) specifically when modified by C-terminal phosphorylation.
Sequence similarities	Belongs to the histone H2A family.
Developmental stage	Synthesized in G1 as well as in S-phase.
Domain	The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.
Post-translational modifications	Phosphorylated on Ser-140 (to form gamma-H2AFX or H2AX139ph) in response to DNA double strand breaks (DSBs) generated by exogenous genotoxic agents and by stalled replication forks, and may also occur during meiotic recombination events and immunoglobulin class switching in lymphocytes. Phosphorylation can extend up to several thousand nucleosomes from the actual

site of the DSB and may mark the surrounding chromatin for recruitment of proteins required for DNA damage signaling and repair. Widespread phosphorylation may also serve to amplify the damage signal or aid repair of persistent lesions. Phosphorylation of Ser-140 (H2AX139ph) in response to ionizing radiation is mediated by both ATM and PRKDC while defects in DNA replication induce Ser-140 phosphorylation (H2AX139ph) subsequent to activation of ATR and PRKDC. Dephosphorylation of Ser-140 by PP2A is required for DNA DSB repair. In meiosis, Ser-140 phosphorylation (H2AX139ph) may occur at synaptonemal complexes during leptotene as an ATM-dependent response to the formation of programmed DSBs by SPO11. Ser-140 phosphorylation (H2AX139ph) may subsequently occurs at unsynapsed regions of both autosomes and the XY bivalent during zygotene, downstream of ATR and BRCA1 activation. Ser-140 phosphorylation (H2AX139ph) may also be required for transcriptional repression of unsynapsed chromatin and meiotic sex chromosome inactivation (MSCI), whereby the X and Y chromosomes condense in pachytene to form the heterochromatic XY-body. During immunoglobulin class switch recombination in lymphocytes, Ser-140 phosphorylation (H2AX139ph) may occur at sites of DNA-recombination subsequent to activation of the activation-induced cytidine deaminase AICDA. Phosphorylation at Tyr-143 (H2AXY142ph) by BAZ1B/WSTF determines the relative recruitment of either DNA repair or pro-apoptotic factors. Phosphorylation at Tyr-143 (H2AXY142ph) favors the recruitment of APBB1/FE65 and pro-apoptosis factors such as MAPK8/JNK1, triggering apoptosis. In contrast, dephosphorylation of Tyr-143 by EYA proteins (EYA1, EYA2, EYA3 or EYA4) favors the recruitment of MDC1-containing DNA repair complexes to the tail of phosphorylated Ser-140 (H2AX139ph). Monoubiquitination of Lys-120 (H2AXK119ub) by RING1 and RNF2/RING2 complex gives a specific tag for epigenetic transcriptional repression. 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.

Cellular localization Nucleus. Chromosome.

Images



Western blot - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

All lanes : Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781) at 1/3000 dilution (purified)

Lane 1 : Raji cell lysate
Lane 2 : HEK293 cell lysate
Lane 3 : Mouse kidney tissue lysate
Lane 4 : Rat kidney tissue lysate

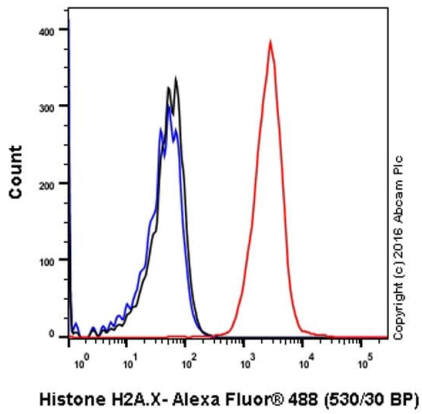
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 15 kDa
Observed band size: 14,22 kDa

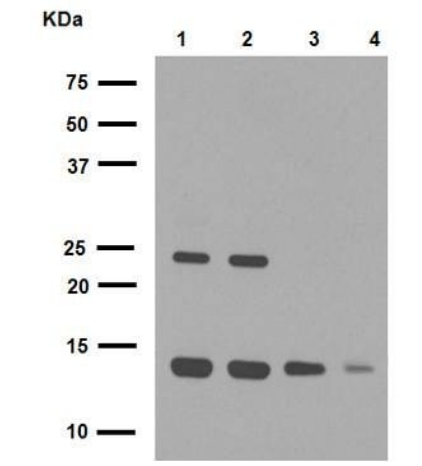
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Flow Cytometry (Intracellular) - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling Histone H2A.X with purified ab124781 at 1/100 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



Western blot - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

All lanes : Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781) at 1/2000 dilution (unpurified)

Lane 1 : Raji cell lysate
Lane 2 : HEK293 cell lysate
Lane 3 : Mouse kidney tissue lysate
Lane 4 : Rat kidney tissue lysate

Lysates/proteins at 20 µg per lane.

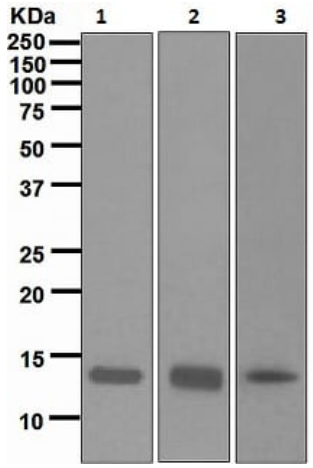
Secondary
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 15 kDa
Observed band size: 14 kDa
Additional bands at: 22 kDa. We are unsure as to the identity of these extra bands.

~22kDa band may be the monoubiquitinated form of histone H2A

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

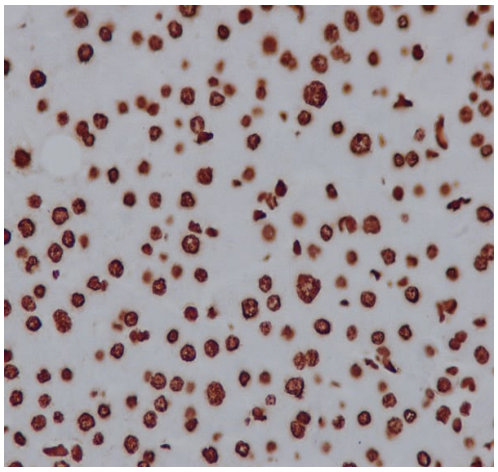
All lanes : Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781) at 1/1000 dilution (unpurified)

Lane 1 : Raji lysate
Lane 2 : Fetal kidney lysate
Lane 3 : Human heart lysate

Lysates/proteins at 10 µg per lane.

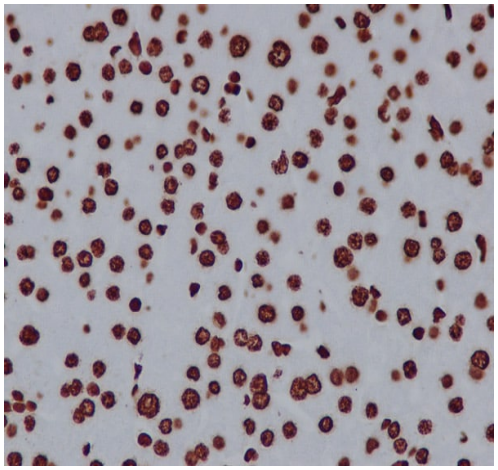
Secondary
All lanes : HRP labelled Goat anti Rabbit IgG at 1/2000 dilution

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



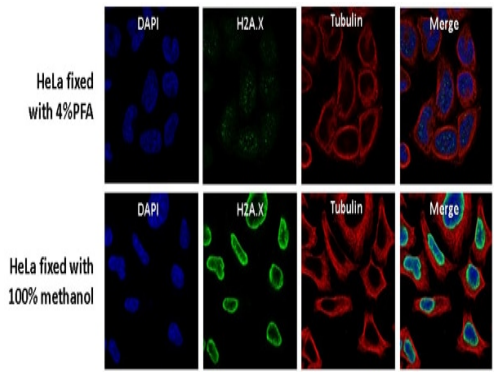
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Histone H2A.X with unpurified ab124781 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with hematoxylin.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

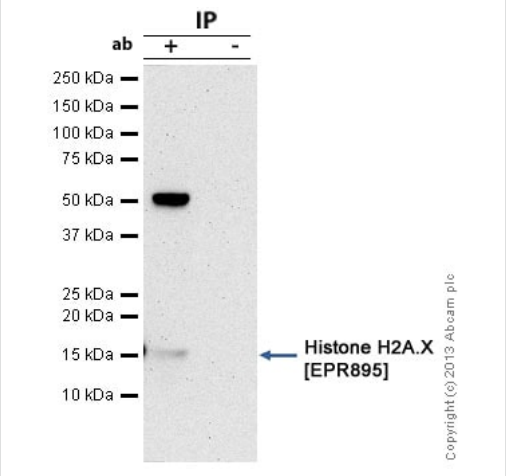
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Histone H2A.X with purified ab124781 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with hematoxylin.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells labelling Histone H2A.X with purified ab124781 at a dilution of 1/1000. Cells were fixed with either 4% PFA (top) or 100% methanol (bottom) and permeabilized with 0.1% TritonX-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody.

ab7291 mouse anti-Tubulin (1/1000) followed by **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were used to label tubulin. DAPI was used as the nulear counterstain.



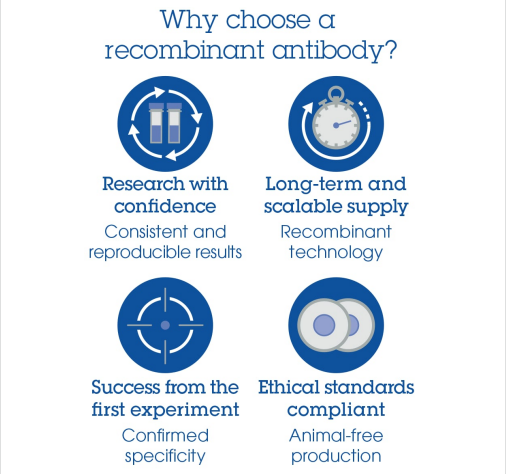
Immunoprecipitation - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

Histone H2A.X was immunoprecipitated using 5ug of ab124781 from 200ul of HeLa whole cell extract lysate diluted to 0.5mg/ml in RIPA and 50ul of Protein G magnetic beads. No antibody was added to the control (-).

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

Proteins were eluted by addition of 40µl SDS loading buffer and incubated for 10min at 70°C. 10µl of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab124781 at 1ug/ml. The Secondary antibody was Mouse monoclonal SB62a Anti-Rabbit IgG light chain (HRP) (**ab99697**) at 1/10,000 dilution.

Band: 15kDa; Histone H2A.X



Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

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