

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

重组 RabMAb

★★★★★ **4 Abreviews** **23 References** **9 图像**

概述

产品名称	Anti-Histone H2A.X抗体[EPR895]-核Marker
描述	兔单克隆抗体[EPR895] to Histone H2A.X -核Marker
宿主	Rabbit
特异性	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.
经测试应用	适用于: WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)
种属反应性	与反应: Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	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).
常规说明	This product is a recombinant monoclonal antibody, which offers several advantages including: <ul style="list-style-type: none"> - 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 .

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide

纯度	Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
克隆	Protein A purified
克隆编号	单克隆
同种型	EPR895
	IgG

应用

The Abpromise guarantee **Abpromise™**承诺保证使用ab124781于以下的经测试应用

“应用说明”部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
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.

靶标

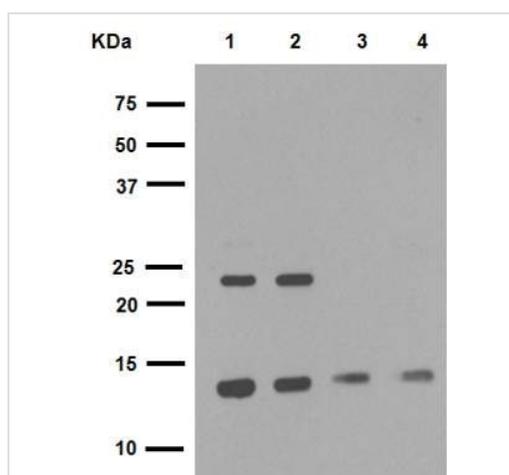
功能	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, DNA 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.
序列相似性	Belongs to the histone H2A family.
发展阶段	Synthesized in G1 as well as in S-phase.
结构域	The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.
翻译后修饰	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.

细胞定位

Nucleus. Chromosome.

图片



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

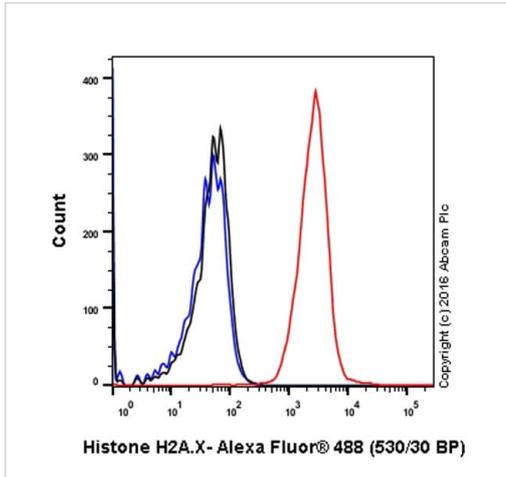
All lanes : Peroxidase-conjugated goat anti-rabbit IgG (H+L) at 1/1000 dilution

Predicted band size: 15 kDa

Observed band size: 14,22 kDa

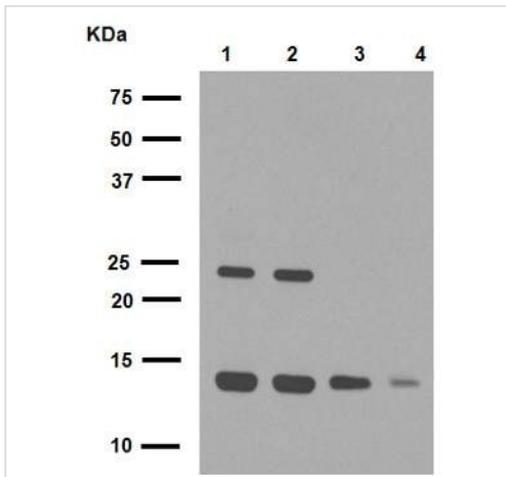
Blocking buffer and concentration: 5% NFD/MTBST.

Diluting buffer and concentration: 5% NFD/MTBST.



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

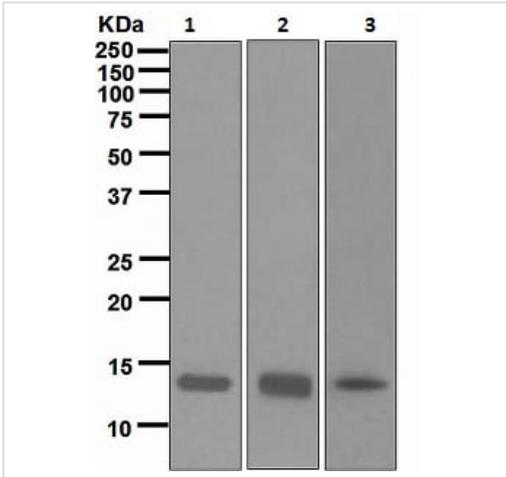
Observed band size: 14 kDa

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% NFD/MTBST.

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

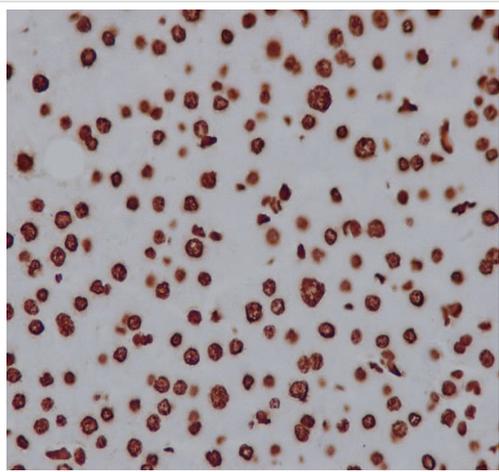
Lysates/proteins at 10 µg per lane.

Secondary

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

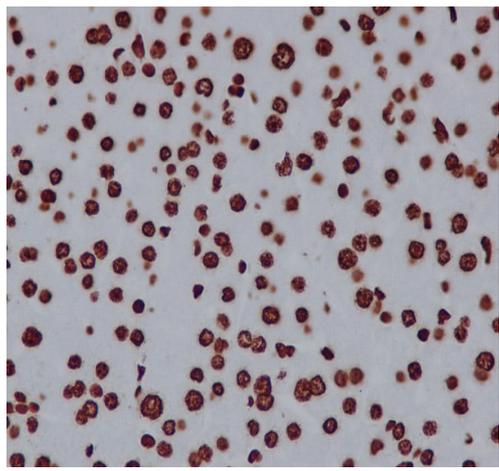
Predicted band size: 15 kDa

Observed band size: 14 kDa



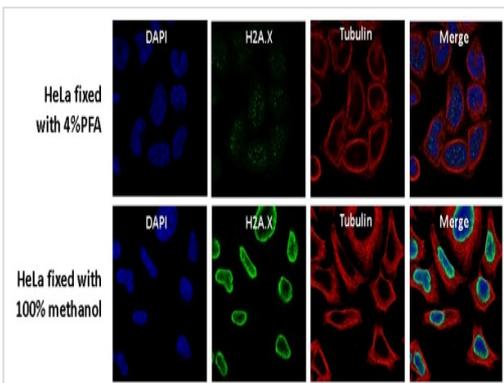
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [EPR895] - Nuclear Marker (ab124781)

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)

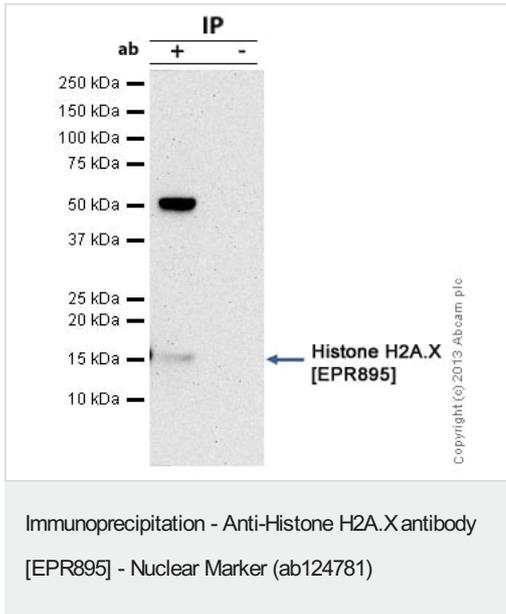
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Histone H2A.X with purified ab124781 at 1/500. Heat mediated antigen retrieval was performed using Tris/EDTA buffer, pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the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



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

Why choose a recombinant antibody?

<p>Research with confidence Consistent and reproducible results</p>	<p>Long-term and scalable supply Recombinant technology</p>
<p>Success from the first experiment Confirmed specificity</p>	<p>Ethical standards compliant Animal-free production</p>

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