abcam

Product datasheet

Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade ab229914

KO 評価済 リコンビナント RabMAb

13 References 画像数 11

製品の概要

製品名	Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade
製品の詳細	Rabbit monoclonal [EPR22820-23] to Histone H2A.X - ChIP Grade
由来種	Rabbit
アプリケーション	**適用あり:** PepArr, ChIP, ICC/IF, Flow Cyt (Intra), WB, IHC-P, IP
種交差性	**交差種:** Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
ポジティブ・コントロール	WB: Wild-type HAP1 whole, Human brain, HeLa, 293T and HEK-293 lysates. IHC-P: Human breast carcinoma and Human testis tissues. ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells. IP: HeLa cells.
特記事項	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**.

製品の特性

製品の状態	Liquid
保存方法	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
バッファー	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
精製度	Protein A purified
ポリ/モノ	モノクローナル

クローン名	EPR22820-23
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab229914の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
PepArr		Use a concentration of 0.1 µg/ml.
ChIP		Use 5 µg for 25 µg of chromatin.
ICC/IF		1/100.
Flow Cyt (Intra)		1/50.
WB		1/1000. Predicted molecular weight: 15 kDa.
IHC-P		1/200.
IP		1/30.

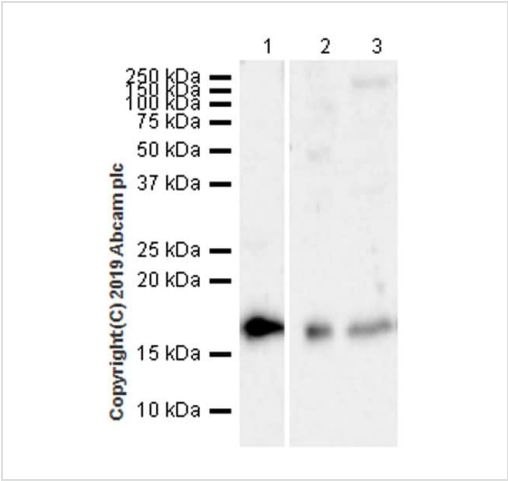
ターゲット情報

機能	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.
配列類似性	Belongs to the histone H2A family.
発生段階	Synthesized in G1 as well as in S-phase.
ドメイン	The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.
翻訳後修飾	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.

細胞内局在 Nucleus. Chromosome.

画像



Western blot - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

All lanes : Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914) at 1/1000 dilution

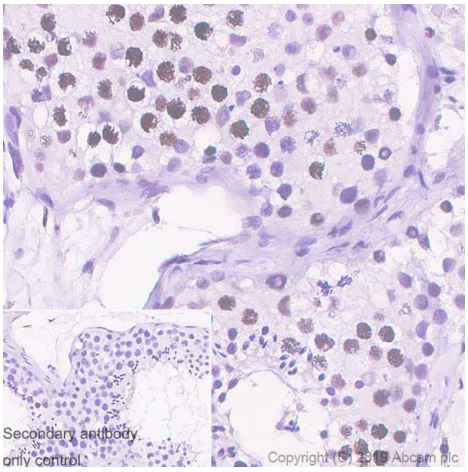
Lane 1 : Human brain tissue lysate
Lane 2 : HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate
Lane 3 : 293T (human embryonic kidney epithelial cell), whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
Lane 1 : VeriBlot for IP secondary antibody (HRP) at 1/1000 dilution
Lanes 2-3 : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 15 kDa
Observed band size: 16,25 kDa

This blot was developed using a higher sensitivity ECL substrate.

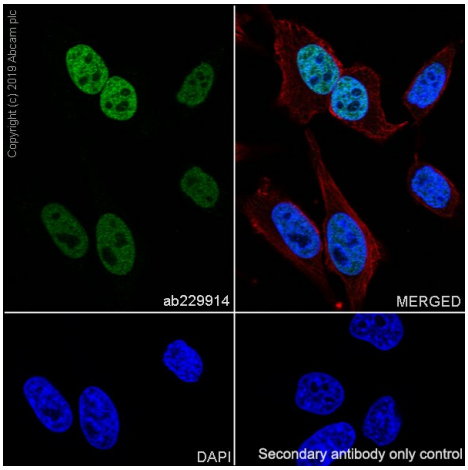


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling Histone H2A.X with ab229914 at 1/200 dilution (2.56 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on the human testis (PMID/ 24059746). The section was incubated with ab229914 for 15 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**)

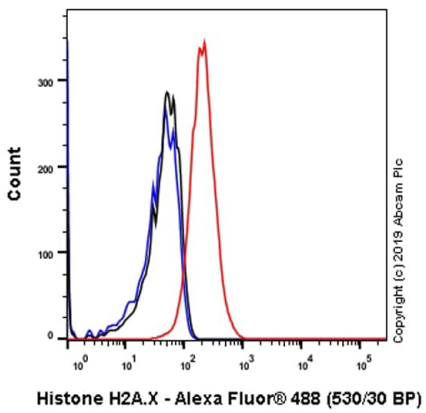
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

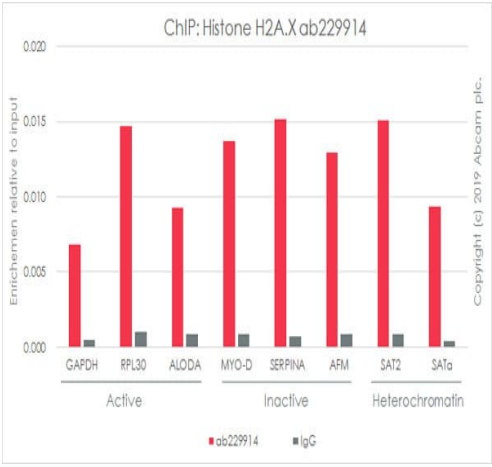
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling Histone H2A.X with ab229914 at 1/100 dilution, followed by Ab229914 anti- Histone H2A.X **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in HeLa cell line is observed. Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling Histone H2A.X with ab229914 at 1/50 dilution (Red), compared with a Rabbit monoclonal IgG (**ab172730**) isotype control (Black)and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor¿½ 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



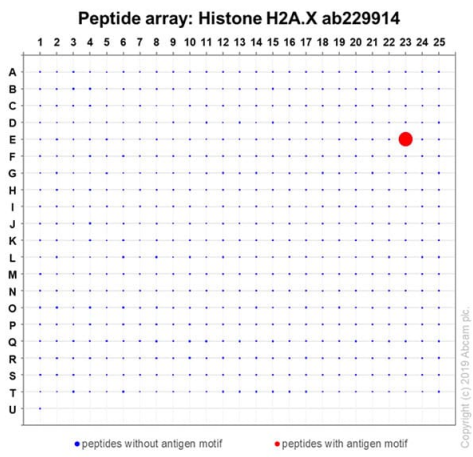
ChIP - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol*. Cells were fixed with formaldehyde for 10min.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab229914 (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci).

Primers and probes are located in the first kb of the transcribed region.

*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol.

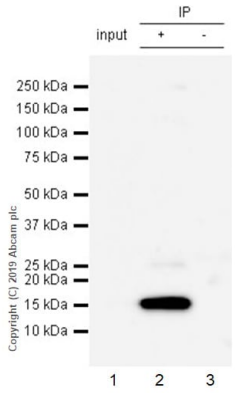


Peptide Array - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

All batches of ab229914 are tested in Peptide Array against 501 different modified and unmodified histone peptides; each peptide is printed on the array at six concentrations (each in triplicate).

Circle area represents affinity between the antibody and a peptide: all antigen-containing peptides are displayed as red circles, all other peptides as blue circles. The affinity is calculated as area under curve when antibody binding values are plotted against the corresponding peptide concentration. Each circle area is normalized to the peptide with the strongest affinity.

The complete dataset, including full list of all peptides and information on the position of each peptide in the diagram, can be downloaded **here**.



Immunoprecipitation - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

Histone H2A.X was immunoprecipitated from 0.35 mg HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate with ab229914 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab229914 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2: ab229914 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab229914 in HeLa whole cell lysate

Blocking and dilution buffer and concentration/ 5% NFDM/TBST.

Exposure time/ 30 seconds.

Western blot - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

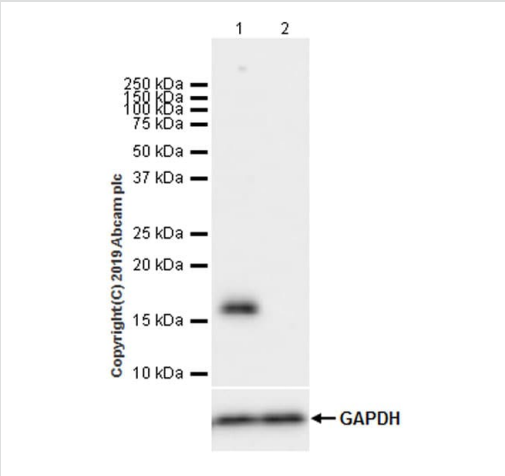
Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914) at 1/1000 dilution + HEK-293 (human embryonic kidney epithelial cell), histone extract at 20 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

Predicted band size: 15 kDa
Observed band size: 16, 25 kDa

A 25-kDa band, likely to be ubiquitinated H2A.X, is observed. The molecular weights are consistent with what have been described in the literature (PMID: 24603765)



Western blot - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

All lanes : Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914) at 1/1000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : Histone H2A.X knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

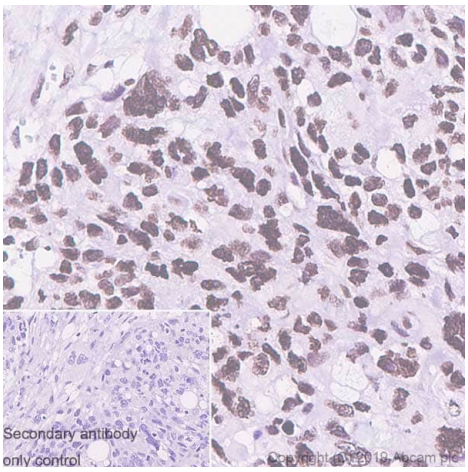
Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 15 kDa
Observed band size: 16 kDa

Ab229914 was shown to specifically react with Histone H2A.X in wild-type HAP1 cells as signal was lost in Histone H2A.X knockout cells. Wild-type and Histone H2A.X knockout samples were

subjected to SDS-PAGE. ab229914 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RADÂ® ChemiDocâ„¢ MP instrument using the ECL technique. Blocking/Diluting buffer and concentration: 5% NFDM/TBST Exposure Time: 37 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling Histone H2A.X with ab229914 at 1/200 dilution (2.56 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on the human breast carcinoma (PMID/ 27006338). The section was incubated with ab229914 for 15 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**)

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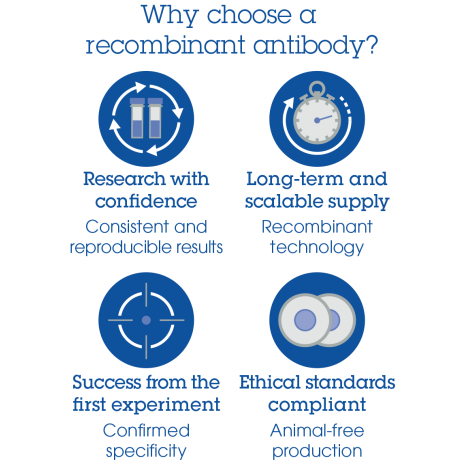
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

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