abcam

ab229914

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

Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914) is a rabbit monoclonal antibody detecting Histone H2A.X in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF, ChIP, PepArr**. Suitable for **Human**.

- KO validated for confirmed specificity
- Biophysical QC for unrivalled batch-batch consistency
- Over 20 publications

Recombinant | RabMAb | Advanced Validation | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR22820-23
Purification technique	Affinity purification Protein A
Concentration	0.503 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

ChIP

Tested

Species	Human
Dilution info	5 µg for 25 µg chromatin
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

PepArr

Tested

Species	Human
Dilution info	0.1 µg/mL
Notes	-

IHC-P

Tested

Species	Human
Dilution info	1/200
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

Target data

See full target information H2AX

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.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot

Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), ChIP, Peptide Array in Human samples.

What is the molecular weight of Histone H2A.X?
Anti-Histone H2A.X [EPR22820-23] - ChIP Grade (ab229914) specifically detects a band for Histone H2A.X (UniProt: P16104) at a molecular weight of 15kDa.

Trusted by the scientific community
Anti-Histone H2A.X [EPR22820-23] - ChIP Grade (ab229914) was first used in a scientific publication in 2019 and has been cited over 20 times in peer-reviewed journals.

Specificity confirmed
The specificity of Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914) has been confirmed by Western blot testing in H2AX Knockout HAP1 cells.

Other related products
We have a range of other formats of antibody clone [EPR22820-23] also available for your convenience: ab229914, Carrier free - ab256544, Alexa Fluor® 647 - ab302969, Alexa Fluor® 488 - ab307464, PE - ab314253

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Trial sizes available!
Test your antibody or perform pre-screening before committing to a larger quantity. Sold in 20µl. Discover our selection of trial-size antibodies.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

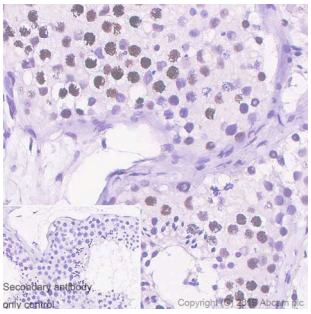
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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10 product images

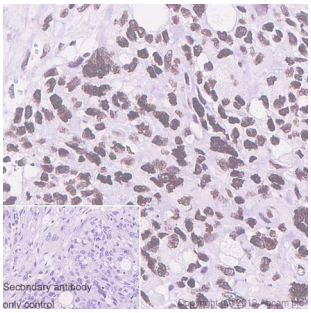


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

Histone H2A.X Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H2A.X antibody

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.



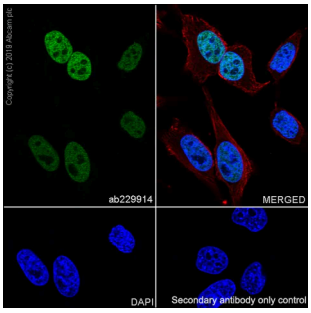
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914)

Histone H2A.X Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Histone H2A.X antibody

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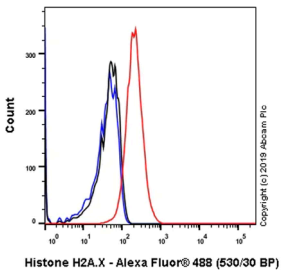
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins.



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

Histone H2A.X Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Histone H2A.X antibody

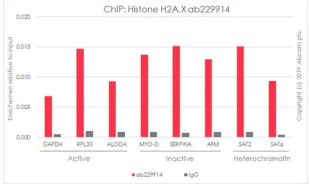
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling Histone H2A.X with ab229914 at 1/100 dilution, followed by ab229914 anti- Histone H2A.X ab150077 AlexaFluor®488 Goat anti-Rabbit secondary antibody 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)

Histone H2A.X Flow Cytometry (Intracellular) staining using rabbit Anti-Histone H2A.X antibody

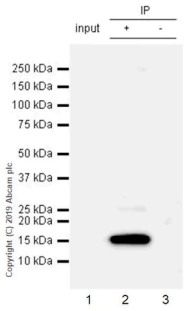
Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling Histone H2A.X with ab229914 at 1/50 dilution (Red), compared with a Rabbit monoclonal IgG (ab172730) isotype control (Black)and an unlabelled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



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

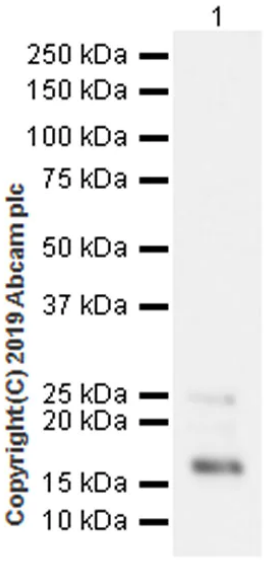
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab229914 in HeLa whole cell lysate

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

Exposure time/ 30 seconds.

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

Predicted band size: 15 kDa



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

Histone H2A.X Western blot staining of HEK-293 (human embryonic kidney epithelial cell), histone extract using rabbit Anti-Histone H2A.X antibody

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

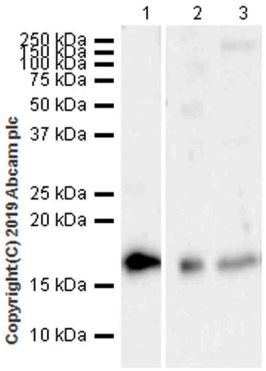
All lanes:
HEK-293 (human embryonic kidney epithelial cell), histone extract at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 15 kDa

Observed band size: 16 kDa, 25 kDa



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

This blot was developed using a higher sensitivity ECL substrate.
All lanes:
Western blot - Anti-Histone H2A.X antibody [EPR22820-23] - ChIP Grade (ab229914) at 1/1000 dilution

Lane 1:
Human brain tissue lysate at 20 µg

Lane 2:
HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 3:
293T (human embryonic kidney epithelial cell), whole cell lysate at 20 µg

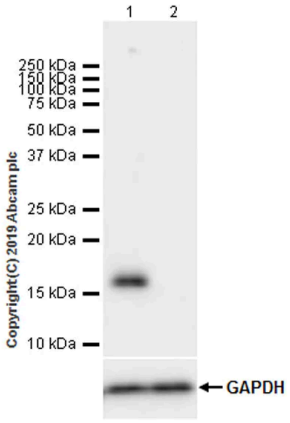
Secondary

Lane 1:
VeriBlot for IP secondary antibody (HRP) at 1/1000 dilution

Lanes 2 - 3:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 15 kDa

Observed band size: 16 kDa, 25 kDa



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

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

Lane 1:
Wild-type HAP1 whole cell lysate at 20 µg

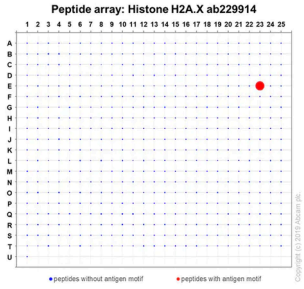
Lane 2:
Histone H2A.X knockout HAP1 whole cell lysate at 20 µg

Secondary

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

Predicted band size: 15 kDa

Observed band size: 16 kDa



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, is available under the Product Protocol section.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.