

Product datasheet

Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade ab81299

Recombinant RabMAb

★★★★☆ 12 Abreviews 53 References 12 Images

Overview

Product name	Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade
Description	Rabbit monoclonal [EP854(2)Y] to gamma H2A.X (phospho S139) - ChIP Grade
Host species	Rabbit
Tested applications	Suitable for: IP, WB, IHC-P, ICC/IF, ChIP, Dot blot
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide (the amino acid sequence is considered to be commercially sensitive) corresponding to Human Histone H2A.X aa 100 to the C-terminus (phospho S139). A synthetic phospho-peptide corresponding to residues surrounding serine 139 of Human Histone H2A.X protein was used as the immunogen Database link: P16104
Positive control	WB: HepG2 and Jurkat cell lysates. IHC-P: Human kidney transitional cell carcinoma, human brain, rat and mouse testis. ICC/IF: H2O2 treated HeLa cells. IP: HepG2 cell lysate.
General notes	Our RabMAb [®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents . We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team. This product is a recombinant rabbit monoclonal antibody .

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. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP854(2)Y
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab81299** 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
IP		1/40.
WB	★★★★☆	1/5000 - 1/10000. Predicted molecular weight: 15 kDa.
IHC-P	★★★★☆	1/50 - 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF	★★★★★	1/250.
ChIP	★★★★★	Use at an assay dependent concentration. PubMed: 20360682
Dot blot		1/1000.

Target

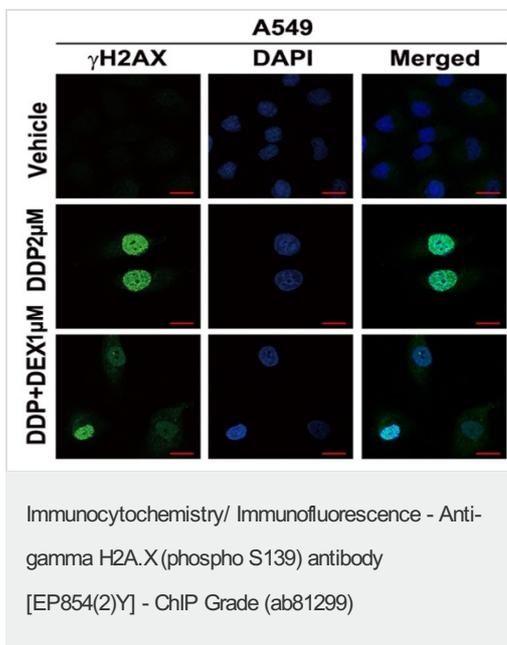
Function	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA repair, 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 occur 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.

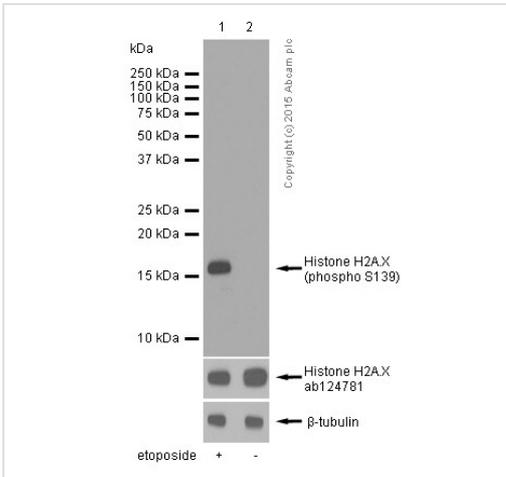
Images



Immunofluorescence staining of A549 (Human lung carcinoma cell line) labeling gamma H2A.X (phospho S139) (green) with ab81299.

Cells were fixed in 4% paraformaldehyde for 15 minutes and permeabilized in PBS-0.2% Triton for 10 minutes. After blocked for 1 hour, primary antibody was diluted in blocking buffer (1/100) and incubated with fixed cells overnight at 4°C. Cells were washed and incubated with secondary antibodies (1/100) for 1 hour at room temperature. All slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI). Immunofluorescence was performed using confocal laser scanning microscopy (Leica) or fluorescence microscopy (Olympus).

Dexamethasone, DEX; Cisplatin, DDP.



Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

All lanes : Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299) at 1/100000 dilution

Lane 1 : HepG2 cell lysate – treated with etoposide

Lane 2 : HepG2 cell lysate – untreated

Lysates/proteins at 20 µg per lane.

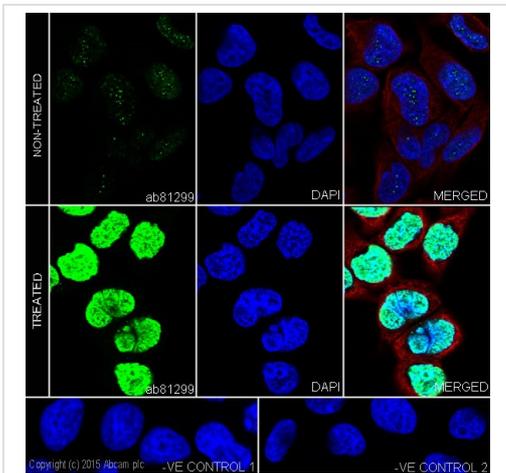
Secondary

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

Predicted band size: 15 kDa

Blocking buffer - 5% NFDM/TBST

Diluting buffer - 1% BSA



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

Immunocytochemistry/Immunofluorescence analysis of HeLa cells (untreated and treated with H₂O₂) labelling Histone H2A.X

(phospho S139) with ab81299 at 1/250. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100.

ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG

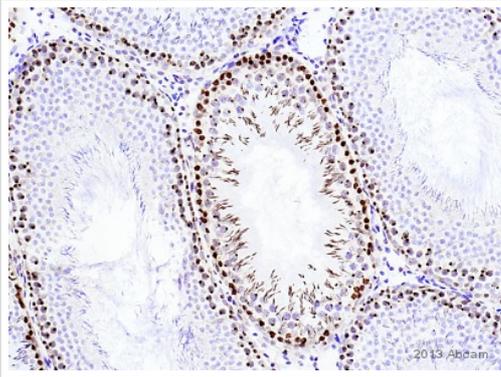
(1/1000) was used as the secondary antibody. The cells were co-stained with ab7291, a mouse anti-tubulin (1/1000)

using ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000) as the secondary antibody. Nuclei counterstained with DAPI (blue).

Control 1: primary antibody (1/250) and secondary antibody,

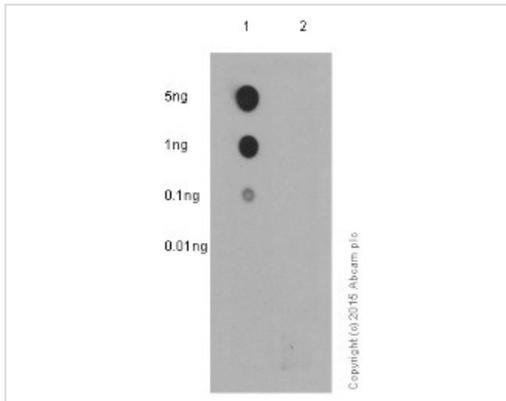
ab150120, an Alexa Fluor[®] 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: ab7291 (1/1000) and secondary antibody, ab150077, an Alexa Fluor[®] 488-conjugated goat anti-rabbit IgG (1/500).



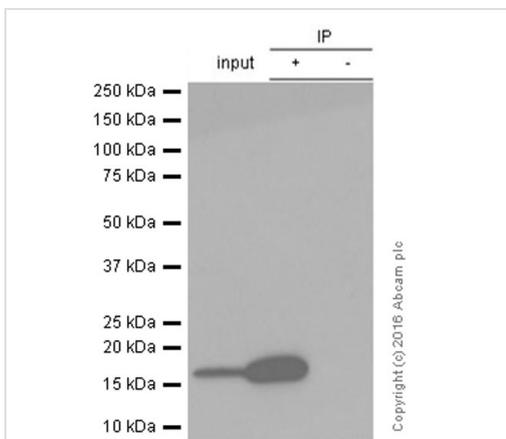
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)
Image courtesy of Carl Hobbs (Kings College London, United Kingdom)

IHC-P image of γ H2A.X staining on Rat testis sections using unpurified ab81299 (1:5000). The sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 mins at 21°C. ab81299 was diluted 1:5000 in TBS buffer (containing BSA and Azide) and sections were then incubated with unpurified ab81299 for 2 hours at 21°C. The secondary antibody used was Biotin conjugated Goat polyclonal to Rabbit IgG (1:250).



Dot Blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

Dot blot analysis of Histone H2A.X single phospho peptide pS139 (lane 1) and Histone H2A.X non-phospho peptide (lane 2) with ab81299 at 1/1000. Blocking and diluting buffer was 5% NFDN/TBST. The secondary antibody used was [ab97051](#) Peroxidase conjugated Goat Anti-Rabbit IgG, (H+L) at 1/100,000.



Immunoprecipitation - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

ab81299 at 1/40 immunoprecipitating Histone H2A.X (phospho S139) in HepG2 (human hepatocellular carcinoma epithelial) whole cell lysate observed at 15 KDa (lanes 1 and 2).

Lane 1 (input): HepG2 treated with etoposide and TSA whole cell lysate 10 μ g

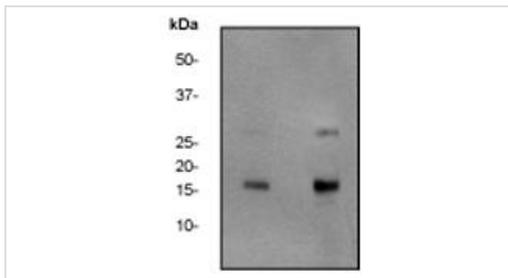
Lane 2 (+): ab81299 + HepG2 treated with etoposide and TSA whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG ([ab172730](#)) instead of ab81299 in HepG2 treated with etoposide and TSA

For western blotting, ab81299 (Purified) at 1/200 dilution and [ab131366](#) VeriBlot for IP (HRP) was used as the secondary antibody (1/1000).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

All lanes : Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299) at 1/500000 dilution (unpurified)

Lane 1 : Jurkat cell lysates

Lane 2 : Jurkat cell lysates treated with etoposide

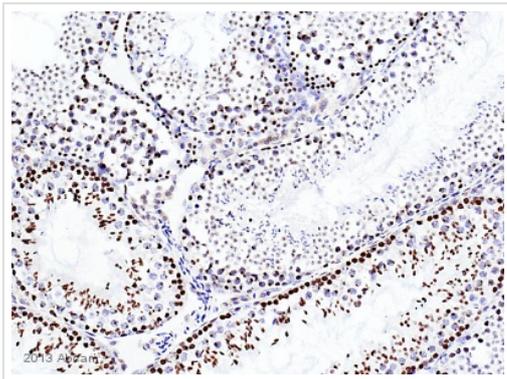
Lysates/proteins at 10 µg per lane.

Secondary

All lanes : HRP Labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 15 kDa

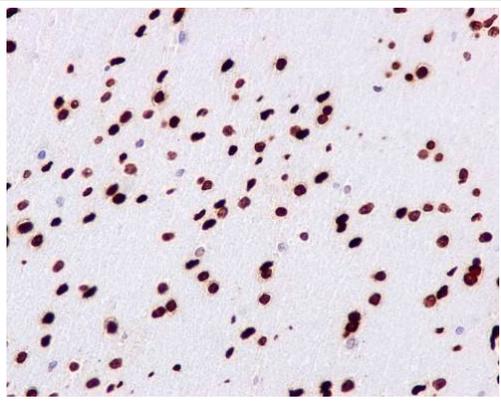
Observed band size: 15 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

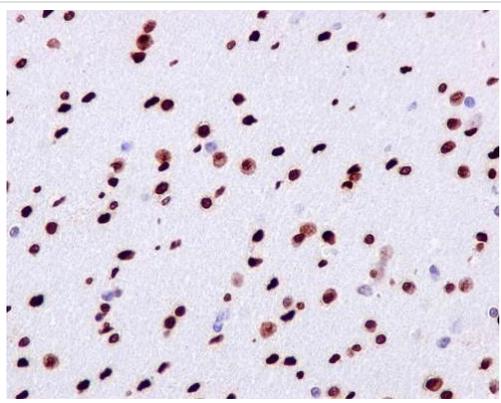
Image courtesy of Carl Hobbs (Kings College London, United Kingdom)

IHC-P image of γ H2A.X staining on Mouse testis sections using unpurified ab81299 (1:5000). The sections were deparaffinized and subjected to heat mediated antigen retrieval using citric acid. The sections were blocked using 1% BSA for 10 mins at 21°C. ab81299 was diluted 1:5000 in TBS buffer (containing BSA and Azide) and sections were then incubated with unpurified ab81299 for 2 hours at 21°C. The secondary antibody used was Biotin conjugated Goat polyclonal to Rabbit IgG (1:250).



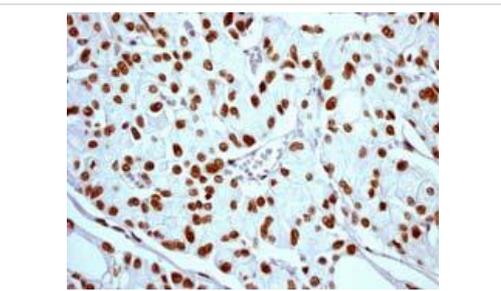
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

Immunohistochemical staining of paraffin embedded human brain with purified ab81299 at a working dilution of 1 in 100. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.



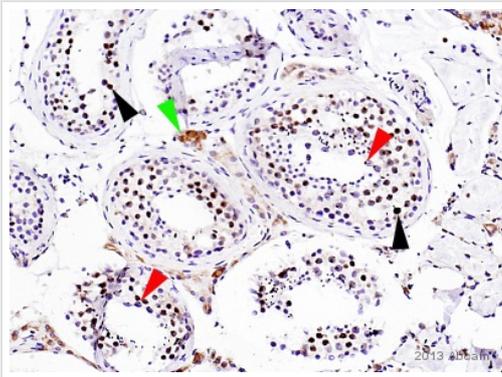
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

Immunohistochemical staining of paraffin embedded human brain with unpurified ab81299 at a working dilution of 1 in 50. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

Immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded human kidney transitional cell carcinoma using unpurified ab81299 at a dilution of 1/100.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] - ChIP Grade (ab81299)

This image is courtesy of an Abreview submitted by Carl Hobbs.

Ab81299 staining H2A.X in Human Testis tissue sections by Immunohistochemistry (Formalin/PFA fixed paraffin embedded sections). Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in Citric acid. Samples were incubated with primary antibody (1/50 in TBS) for 2 hours at 21°C. A biotin conjugated Anti-Rabbit IgG (goat polyclonal) was used as the secondary antibody at a 1/250 dilution.

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