

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

Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) is a rabbit monoclonal antibody detecting gamma H2A.X in **Western Blot, IP, IHC-P, ICC/IF, Dot Blot**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 270 publications
- Trusted since 2009

Recombinant

RabMAb

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	EP854(2)Y
Purification technique	Affinity purification Protein A
Concentration	0.71 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Human
Dilution info	1/40
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Sheep
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	1/1000
Notes	-

Expected

Species	Human, Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Sheep
Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	1/1000 - 1/500000
Notes	-

Species	Mouse
Dilution info	1/1000 - 1/500000
Notes	-

Species	Rat
Dilution info	1/1000 - 1/500000
Notes	-

Predicted

Species	Sheep
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat, Sheep, Synthetic peptide - Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Sheep
Dilution info	-
Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-

Target data

[See full target information H2AX_pS139](#) 

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.
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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

Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Dot Blot, ICC/IF, IHC-P, IP and WB.

Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) was first used in a scientific publication in 2008 and has been cited over 272 times in peer reviewed journals. Its performance in Western Blot and immunofluorescence in human samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) has 16 independent reviews from customers.

Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) specifically detects gamma H2A.X Phospho-S139 (UniProt ID: P16104; Molecular weight: 16kDa) and is sold in a convenient trial size to enable initial testing (20 µL) and larger sizes for subsequent scaling up experiments (100 µL and 1 mL).

Conjugation-ready, carrier free format available for antibody clone EP854(2)Y - ab215967.

Antibody clone EP854(2)Y is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, HRP, Alexa Fluor® 594, Alexa Fluor® 555 (ab195188, ab195189, ab195190, ab206898, ab206900).

Gamma H2A.X (γH2A.X) is a phosphorylated form of the H2A histone family member X (H2AX), which plays a crucial role in the cellular response to DNA damage. When double-strand breaks occur in DNA, H2AX is rapidly phosphorylated at serine 139 to form γH2A.X. This modification serves as a marker for DNA damage and is essential for recruiting DNA repair proteins to the site of damage. Researchers use γH2A.X as a sensitive indicator of DNA double-strand breaks in studies of genotoxic stress, cancer and other conditions involving DNA damage and repair.

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary

Gamma H2A.X also known as phospho H2A.X or γH2A.X is a phosphorylated form of the histone variant H2A.X. It has a molecular weight of about 14 kilodaltons and occurs primarily in the nucleus. When DNA double-strand breaks (DSBs) occur, serine 139 in H2A.X undergoes rapid phosphorylation resulting in gamma H2A.X. This modification happens swiftly at the site of damage and gamma H2A.X spreads over a large chromatin area facilitating the recruitment of DNA repair proteins. Gamma H2A.X staining typically evaluated using gamma H2A.X immunofluorescence techniques aids in identifying the presence and extent of DNA damage.

Biological function summary

Gamma H2A.X plays a role in DNA damage response and repair. It does not operate alone; it acts as part of a complex with other repair proteins. The formation of gamma H2A.X foci at DNA damage sites creates a signal attracting repair factors that help maintain genome stability. Its interaction with MDC1 and ATM proteins exemplifies its significant role in orchestrating an effective response to DNA damage. Beyond DNA repair, gamma H2A.X influences cell cycle checkpoints, permitting cells to pause and repair before proceeding with division.

Pathways	Gamma H2A.X plays a pivotal role in the DNA damage response (DDR) pathway. This pathway is essential for detecting and repairing DNA lesions to uphold genomic integrity. Within the DDR pathway gamma H2A.X is closely associated with proteins such as NBS1 and BRCA1 which assist in repairing double-strand breaks. In addition gamma H2A.X is integral to the ATM-ATR signaling pathway where its activation promotes cell survival following genotoxic stress by signaling for damage repair or triggering apoptosis.
Associated diseases and disorders	Gamma H2A.X has connections to cancer and neurodegeneration. Aberrant DNA repair pathways often indicated by persistent gamma H2A.X signals correlate with tumor formation and progression. For instance a failure to repair DNA damage effectively can lead to mutations that drive cancer development. Gamma H2A.X also links to neurodegenerative diseases where dysregulated DNA repair contributes to neuronal cell death. Proteins like p53 which regulate cell cycle and apoptosis further connect to gamma H2A.X bridging its role in disease pathogenesis.

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

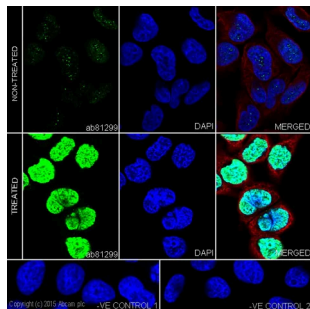
We do not recommend this combination. It is not covered by our product promise.

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](#).

17 product images



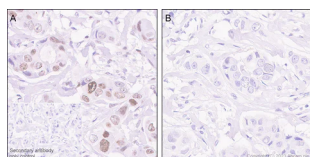
Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299)

Gamma H2A.X (phospho S139) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

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).

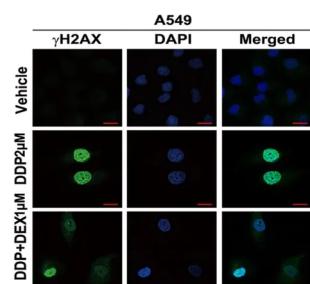
Control 2: [ab7291](#) (1/1000) and secondary antibody, [ab150077](#), an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/500).



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

Gamma H2A.X (phospho S139) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human breast carcinoma using rabbit Anti-gamma H2A.X (phospho S139) antibody

Immunohistochemical analysis of paraffin-embedded Human breast carcinoma tissue labeling gamma H2A.X with ab81299 at 1/3000 (0.352 ug/ml). Nuclear staining on human breast carcinoma without lambda protein phosphatase treatment (image A). No signal was detected when tissues were treated with lambda protein phosphatase (image B). The section was incubated with ab81299 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 [ab209101](#). Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.

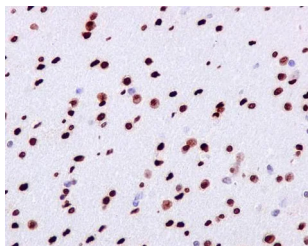


Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299)

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 (Lecia) or fluorescence microscopy (Olympus).

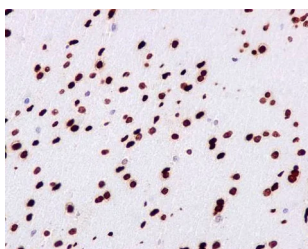
Dexamethasone, DEX; Cisplatin, DDP.



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

Gamma H2A.X (phospho S139) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

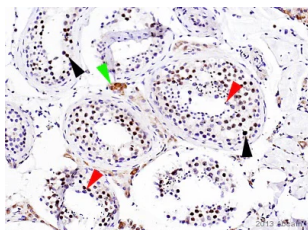
Immunohistochemical staining of paraffin embedded human brain with unpurified ab81299 at a working dilution of 1 in 50. The secondary antibody used is a HRP polymer for rabbit IgG. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0.



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

Gamma H2A.X (phospho S139) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

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.

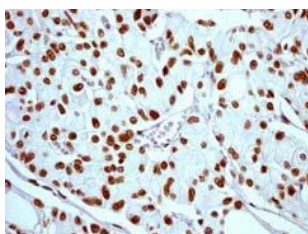


This image is courtesy of a customer review submitted by Carl Hobbs.

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

Gamma H2A.X (phospho S139) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

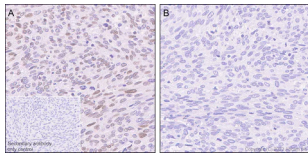
ab81299 staining H2A.X in Human Testis tissue sections by Immunohistochemistry (Formalin/PFA fixed paraffin embedded sections). Tissue was fixed with formaldehyde and blocked with 1% BSA for 10 minutes at 21°C; antigen retrieval was by heat mediation in Citric acid. Samples were incubated with primary 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.



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

Gamma H2A.X (phospho S139) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

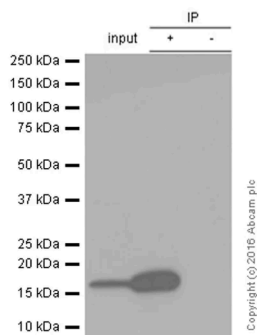
Immunohistochemical analysis of formalin/PFA-fixed paraffin-embedded human kidney transitional cell carcinoma using unpurified ab81299 at a dilution of 1/100. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

Gamma H2A.X (phospho S139) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Human cervical carcinoma using rabbit Anti-gamma H2A.X (phospho S139) antibody

Immunohistochemical analysis of paraffin-embedded Human cervical carcinoma tissue labeling gamma H2A.X with ab81299 at 1/3000 (0.352 ug/ml). Nuclear staining on human cervical carcinoma without lambda protein phosphatase treatment (image A). No signal was detected when tissues were treated with lambda protein phosphatase (image B). The section was incubated with ab81299 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 [ab209101](#). Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins.



Immunoprecipitation - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (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 10µg

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 VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/1000 dilution.

Blocking buffer and concentration: 5% NFDm/TBST.

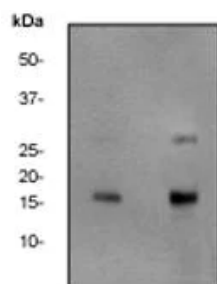
Diluting buffer and concentration: 5% NFDm /TBST.

All lanes:

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

Predicted band size: 15 kDa

Exposure time: 30s



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

All lanes:

Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) at 1/500000 dilution

Lane 1:

Jurkat cell lysate - untreated at 10 µg

Lane 2:

Jurkat cell lysate - treated with etoposide at 10 µg

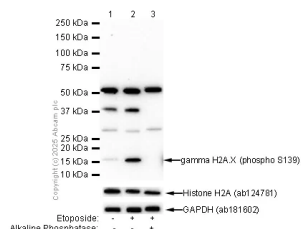
Secondary

All lanes:

HRP Labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 15 kDa

Observed band size: 15 kDa



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

Gamma H2A.X (phospho S139) Western blot staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control ([ab181602](#)) staining at 1/1000000 dilution.

In Western blot, Anti-Histone H2A antibody [EPR895] - Nuclear Marker ([ab124781](#)) staining at 1/2000 dilution.

The identity of the bands between 25kDa and 50kDa are unknown.

All lanes:

Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) at 1/1000 dilution

Lane 1:

Untreated Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 μg

Lane 2:

Jurkat treated with 25μM etoposide for 8 hours whole cell lysate at 20 μg

Lane 3:

Jurkat treated with 25μM etoposide for 8 hours whole cell lysate 20μg, then the membrane treated with Alkaline Phosphatase for 1 hour at 20 μg

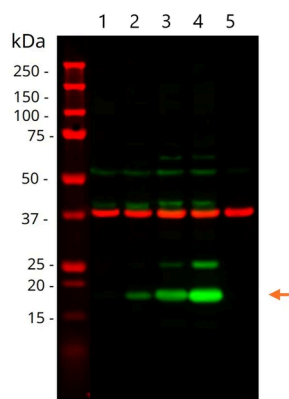
Secondary

All lanes:

Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Observed band size: 15 kDa

Exposure time: 60s



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

Gamma H2A.X (phospho S139) Western blot staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

Mouse anti GAPDH was used as a GAPDH loading control.

All lanes:

Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) at 1/1000 dilution

Lane 1:

HepG2 Vehicle Control Etoposide (0 uM, 90 min) at 20 μg

Lane 2:
HepG2 Etoposide treated (100 uM, 90 min) at 20 µg

Lane 3:
Jurkat Vehicle Control Etoposide (0 uM, 90 min) at 20 µg

Lane 4:
Jurkat Treated Etoposide (100 uM, 90 min) at 20 µg

Lane 5:
Human Brain at 20 µg

Predicted band size: 15 kDa

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

Gamma H2A.X (phospho S139) Western blot staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

Western blot: Rabbit Monoclonal [EP854(2)Y] to gamma H2A.X (phospho S139) ab81299 staining at 1/1000 dilution, shown in green; Mouse anti GAPDH ([ab8245](#)) loading control staining at 1/20,000 dilution, shown in magenta. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% BSA in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20,000 dilution.

All lanes:
Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) at 1/1000 dilution

Lane 1:
HepG2 Vehicle Control Etoposide (0 uM, 90 min) at 20 µg

Lane 2:
HepG2 Etoposide treated (100 uM, 90 min) at 20 µg

Lane 3:
Jurkat Vehicle Control Etoposide (0 uM, 90 min) at 20 µg

Lane 4:
Jurkat Treated Etoposide (100 uM, 90 min) at 20 µg

Lane 5:
Human Brain at 20 µg

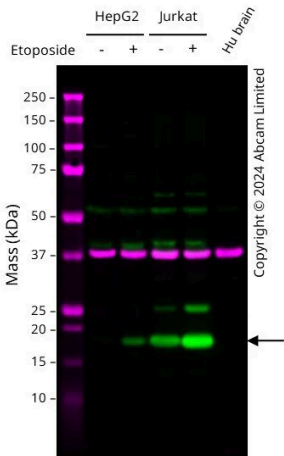
Secondary

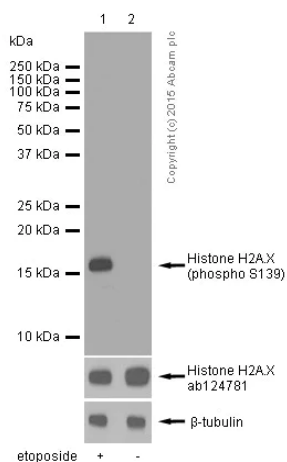
All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 15 kDa

Observed band size: 15 kDa





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

Gamma H2A.X (phospho S139) Western blot staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

Blocking buffer - 5% NFDM/TBST

Diluting buffer - 1% BSA

All lanes:

Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) at 1/100000 dilution

Lane 1:

HepG2 cell lysate – treated with etoposide at 20 µg

Lane 2:

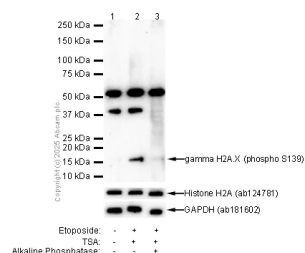
HepG2 cell lysate – untreated at 20 µg

Secondary

All lanes:

Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Predicted band size: 15 kDa



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

Gamma H2A.X (phospho S139) Western blot staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control ([ab181602](#)) staining at 1/1000000 dilution.

In Western blot, Anti-Histone H2A antibody [EPR895] - Nuclear Marker ([ab124781](#)) staining at 1/2000 dilution.

The identity of the bands between 37kDa and 50kDa are unknown.

All lanes:

Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] (ab81299) at 1/1000 dilution

Lane 1:

Untreated NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate at 20 µg

Lane 2:

NIH/3T3 treated with 30µg/ml etoposide for 4 hours, 500ng/ml Trichostatin A(TSA) was then added for additional 4 hours whole cell lysate at 20 µg

Lane 3:

NIH/3T3 treated with 30µg/ml etoposide for 4 hours, 500ng/ml Trichostatin A(TSA) was then added for additional 4 hours whole cell lysate 20µg, then the membrane treated with Alkaline Phosphatase for 1 hour at 20 µg

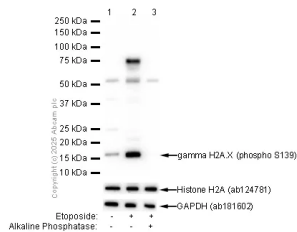
Secondary

All lanes:

Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Observed band size: 15 kDa

Exposure time: 180s



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

Gamma H2A.X (phospho S139) Western blot staining using rabbit Anti-gamma H2A.X (phospho S139) antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control ([ab181602](#)) staining at 1/1000000 dilution.

In Western blot, Anti-Histone H2A antibody [EPR895] - Nuclear Marker ([ab124781](#)) staining at 1/2000 dilution.

The identity of the bands between 20kDa and 75kDa are unknown.

All lanes:

Western blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] ([ab81299](#)) at 1/1000 dilution

Lanes 1 - 2:

PC-12 treated with 3µM etoposide for 1-hour whole cell lysate at 20 µg

Lane 3:

PC-12 treated with 3µM etoposide for 1-hour whole cell lysate 20µg, then the membrane treated with Alkaline Phosphatase for 1 hour at 20 µg

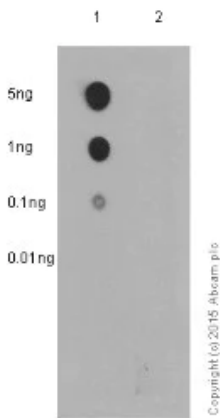
Secondary

All lanes:

Western blot - Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/20000 dilution

Observed band size: 15 kDa

Exposure time: 3s



Dot Blot - Anti-gamma H2A.X (phospho S139) antibody [EP854(2)Y] ([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% NFDM/TBST. The secondary antibody used was [ab97051](#) Peroxidase conjugated Goat Anti-Rabbit IgG, (H+L) at 1/100,000.

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