abcam

ab26350

Anti-gamma H2A.X (phospho S139) antibody [9F3]

Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) is a mouse monoclonal antibody detecting gamma H2A.X in **Western Blot, Flow Cytometry, IHC-P**. Suitable for **Chinese hamster, Human, Mouse, Rat**.

- KO validated for confirmed specificity
- Over 340 publications
- Trusted since 2005

Key facts

Isotype	IgG
Host species	Mouse
Storage buffer	Preservative: 0.09% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine)
Form	Liquid
Clonality	Monoclonal
Immunogen	Synthetic Peptide within Human H2AX pS139. The exact immunogen used to generate this antibody is proprietary information. Database link P16104
Clone number	9F3
Purification technique	Affinity purification Protein G
Specificity	IHC-P is only guaranteed for mouse samples.
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purified from TCS.

Reactivity data

Flow Cyt

Tested

Species	Human
Dilution info	-
Notes	-

Expected

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

WB

Tested

Species	Chinese hamster, Mouse, Rat, Human
Dilution info	-
Notes	-

IHC-P

Expected

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

Not recommended

Species	Human, Rat, Chinese hamster
Dilution info	-
Notes	-

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.

Storage

Shipped at conditions	Blue Ice
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

Notes

Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) is a House Mouse Monoclonal antibody and is validated for use in Flow Cyt, IHC-P, WB.

Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) has been cited over 343 times in peer reviewed journals and is trusted by the scientific community.

Abcams high quality validation processes ensure Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) has high sensitivity and specificity.

Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) has 30 independent reviews from customers.

Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) specifically detects gamma H2A.X Phospho-S139 (UniProt ID: P16104; Molecular weight: 15kDa) and is sold in 100 ug selling sizes.

This product was changed from ascites to tissue culture supernatant on 22nd May 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

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 they 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.

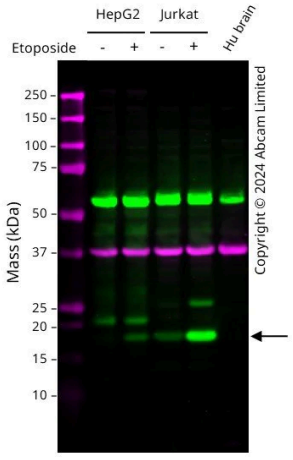
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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8 product images



Western blot - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

Western blot: Mouse Monoclonal [9F3] to gamma H2A.X phospho S139 ab26350 staining at 1/1000 dilution, shown in green; Rabbit anti GAPDH (ab181602) loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 15 kDa in HepG2 Vehicle Control Etoposide (0 uM, 90 min) cell lysates with no signal observed at this size in HepG2 Treated Etoposide (100 uM, 90 min) cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3pc 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-Mouse 800CW & Goat anti-Rabbit 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) at 1/1000 dilution

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

Lane 2:
HepG2 Treated Etoposide (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

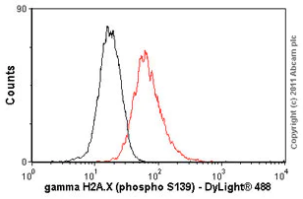
Secondary

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

Performed under reducing conditions.

Predicted band size: 15 kDa

Observed band size: 15 kDa

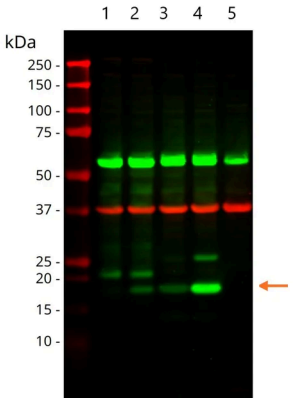


Flow Cytometry - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

Gamma H2A.X (phospho S139) Flow Cytometry staining using mouse Anti-gamma H2A.X (phospho S139) antibody

Overlay histogram showing HeLa cells stained with ab26350 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab26350, 1µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was a mix of mouse IgG1 [ICIGG1], (ab91353, 1µg/1x10^6 cells), IgG2a [ICIGG2A], (ab91361, 1µg/1x10^6 cells), IgG2b [PLPV219], (ab91366, 1µg/1x10^6 cells), IgG3 [MG3-35], (ab18394, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

This image was generated using the ascites version of the product.



Western blot - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

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

Rabbit anti GAPDH was used as a GAPDH loading control. The intensity of the band has increased after treatment in both HepG2 and Jurkat cells.
All lanes:
Western blot - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) at 1/1000 dilution

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

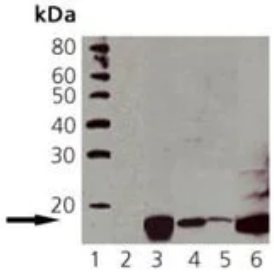
Lane 2:
HepG2 Treated Etoposide (100 uM, 90 min) at 20 µg

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 [9F3] (ab26350)

This image was generated using the ascites version of the product.
All lanes:
Western blot - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

Lane 1:
Molecular weight marker

Lane 2:
Cell lysates prepared from Jurkat (Human T cell leukemia cell line from peripheral blood) cells

Lane 3:
Cell lysates prepared from Jurkat cells treated with staurosporine

Lane 4:
Cell lysates prepared from NIH/3T3 (Mouse embryonic fibroblast cell line) cells

Lane 5:
Cell lysates prepared from CHO-K1 (Chinese hamster ovary cell line) cells

Lane 6:
Cell lysates prepared from Rat2 (Rat fibroblast cell line) cells

Predicted band size: 15 kDa

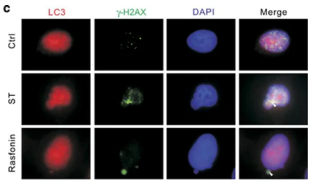


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Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

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

H2A.X immunocytochemistry-immunofluorescence using Anti-gamma H2A.X (phospho S139) antibody [9F3] ab26350. Publication image and figure legend from Yan, S., Liu, L., et al., 2017, Cell Death Dis, 8 (8), Pubmed 28796254.

LC3 interacts with γ-H2AX and Rad51. (a and b) 786-O cells were treated with ST (0-8 μm) or rasfonin (0-6 μm) for 3 h, cells were lysed and subjected to immunoblotting with the antibodies indicated. (c) 786-O cells were treated with ST or rasfonin for 3 h, and the images were obtained using fluorescence microscopy following staining with the antibodies of LC3 and γ-H2AX. (d-f) 786-O cells were incubated with ST or rasfonin for 3 h and lysed, and LC3s or pULK1s were precipitated using the antibody against LC3 or pULK1 (Ser555). The immunoprecipitates were resolved by electrophoresis and probed by immunoblotting with the indicated antibodies. All data were acquired from three independent experiments

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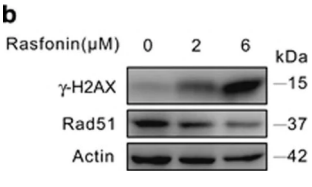


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Western blot - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

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

gamma H2A.X western Blotting using Anti-gamma H2A.X (phospho S139) antibody [9F3] ab26350. Publication image and figure legend from Yan, S., Liu, L., et al., 2017, Cell Death Dis, PubMed 28796254.

LC3 interacts with γ-H2AX and Rad51. (a and b) 786-O cells were treated with ST (0-8 μm) or rasfonin (0-6 μm) for 3 h, cells were lysed and subjected to immunoblotting with the antibodies indicated. (c) 786-O cells were treated with ST or rasfonin for 3 h, and the images were obtained using fluorescence microscopy following staining with the antibodies of LC3 and γ-H2AX. (d-f) 786-O cells were incubated with ST or rasfonin for 3 h and lysed, and LC3s or pULK1s were precipitated using the antibody against LC3 or pULK1 (Ser555). The immunoprecipitates were resolved by electrophoresis and probed by immunoblotting with the indicated antibodies. All data were acquired from three independent experiments

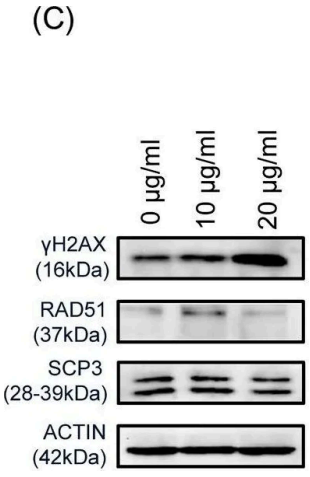


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Western blot - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

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

-gamma H2A.X western blotting using Anti-gamma H2A.X (phospho S139) antibody [9F3] ab26350. Publication image and figure legend from Zhai, Q. Y., Ge, W., et al., 2018, Aging (Albany NY), PubMed 30153657.

nZnO induces DNA damage in fetal oocytes in vitro. (A) Representative double IF of pachytene and diplotene oocytes for SCP3 (red) and γH2AX (green) obtained from ovarian tissues cultured for 6 days. (B) Quantification of oocytes showing distinct γH2AX staining. (C) Representative WB for the indicated proteins from ovarian tissues cultured for 6 days; actin was used as loading control. (D) Representative double IF of pachytene and diplotene oocytes for SCP3 (red) and RAD51 (green). (E) Quantification of oocytes showing RAD51 foci.

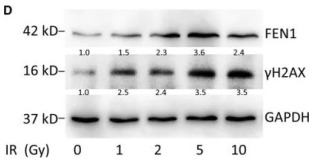


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Western blot - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

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

gamma H2A.X western blotting using Anti-gamma H2A.X (phospho S139) antibody [9F3] ab26350. Publication image and figure legend from Li, J. L., Wang, J. P., et a., 2019, Cancer Med, PubMed 31670906.

FEN1 is overexpressed in cervical cancer and upregulated by ionizing radiation (IR) induction. A, Expression of FEN1 in cervical cancer samples (Tumor) vs control tissues (Ctrl), using the TCGA cervical cancer dataset. B, Scatter plots showing expression of FEN1 and γH2AX in cervical cancer samples. C, Cervical cancer samples were stratified based on median FEN1 expression. GSEA shows enrichment of the GNF2_H2AFX signature in FEN1High samples vs FEN1Low samples. D, FEN1 and γH2AX expression levels in HeLa cells were determined after 2 h of IR treatment. E FEN1 and γH2AX expression levels at different time point in HeLa cells were determined after IR (5 Gy) treatment

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