abcam

ab303656

Anti-gamma H2A.X (phospho S139) antibody [N1-431]

Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656) is a mouse monoclonal antibody detecting gamma H2A.X in **Western Blot, Flow Cytometry (Intra), ICC/IF, Dot Blot, PepArr**. Suitable for **Human, Mouse**.

Recombinant | Advanced Validation

Key facts

Isotype	IgG1
Host species	Mouse
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	N1- 431
Purification technique	Affinity purification Protein A
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human

Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
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
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500

Notes	-

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/1000
Notes	-

Expected

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

Not recommended

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

PepArr

Tested

Species	Human
Dilution info	0.05 µg/mL
Notes	-

Expected

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

Not recommended

Species	Synthetic peptide - Human
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
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656) is a mouse recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Intra), Flow Cytometry (Flow Cyt), Immunocytochemistry/immunofluorescence (ICC/IF), Dot Blot, Peptide Array in Human, Mouse samples.

What is the molecular weight of gamma H2A.X?
Anti-gamma H2A.X (phospho S139) [N1-431] (ab303656) specifically detects a band for gamma H2A.X (UniProt: P16104) at a molecular weight of 15kDa.

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.

Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

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

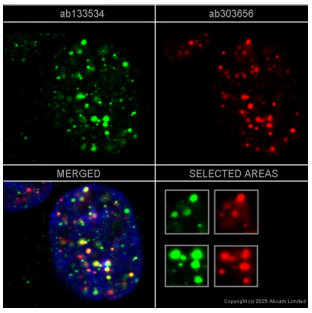
We do not recommend this combination. It is not covered by our product promise.

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.

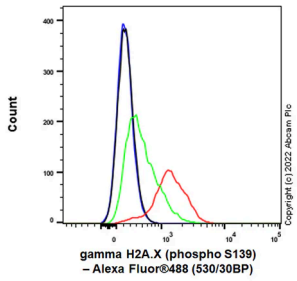
7 product images



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656)

Gamma H2A.X (phospho S139) Immunocytochemistry/ Immunofluorescence staining of UV-treated HCT116 cells using mouse Anti-gamma H2A.X (phospho S139) antibody

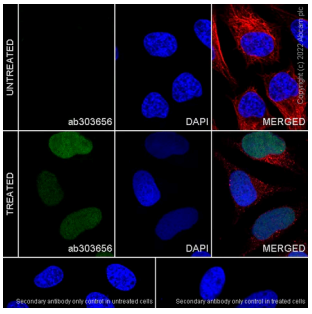
Immunofluorescent analysis of HCT116 cells treated with 20 J/m^2 UV energy 24 hours after seeding, followed by a further 24-hour incubation prior to fixation. Cells were fixed with 4% PFA and permeabilized with 0.1% Triton X-100. Rad51 was labelled with ab133534 at 0.2 µg/ml (shown in green). ab303656 Anti-gamma H2A.X (phospho S139) antibody [N1-431] was used as a DNA damage counterstain at 0.2 µg/ml (shown in red). The secondary antibodies used were ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed and ab150120 Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed, both used at a 1/1000 dilution (2 µg/ml). The image shows selected areas of overlapping foci. The nuclear counterstain was DAPI.



Flow Cytometry (Intracellular) - Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656)

Gamma H2A.X (phospho S139) Flow Cytometry (Intracellular) staining of HeLa (human cervical adenocarcinoma epithelial cell) using mouse Anti-gamma H2A.X (phospho S139) antibody

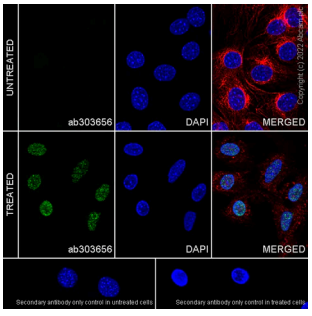
Flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human cervical adenocarcinoma epithelial cell) treated with UV-C (50 mJ/cm2), then recovery (1h) (Red) / Untreated control (Green) cells labeling gamma H2A.X (phospho S139) with ab303656 at 1/1000 dilution (0.1µg), compared with a mouse monoclonal IgG (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti-Mouse IgG (Alexa Fluor® 488, ab150113) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656)

Gamma H2A.X (phospho S139) Immunocytochemistry/ Immunofluorescence staining of HeLa (human cervical adenocarcinoma epithelial cell) using mouse Anti-gamma H2A.X (phospho S139) antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human cervical adenocarcinoma epithelial cell) cells labeling gamma H2A.X (phospho S139) with ab303656 at 1/500 dilution, followed by ab150117 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 µg/ml) (Green). Confocal image showing increased nuclear staining in HeLa cells treated with UV-C (50 mJ/cm2), then recovery (1h). ab202272 Recombinant Alexa Fluor® 594 Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab202272) was used to counterstain tubulin at 1/50 dilution (10 µg/mL) (Red). The nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150117 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (2 µg/ml).

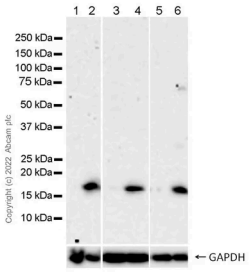


Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656)

Gamma H2A.X (phospho S139) Immunocytochemistry/ Immunofluorescence staining of NIH/3T3 (mouse embryonic fibroblast) using mouse Anti-gamma H2A.X (phospho S139) antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast) cells labeling gamma H2A.X (phospho S139) with ab303656

at 1/500 dilution, followed by ab150117 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 µg/ml) (Green). Confocal image showing increased nuclear staining in NIH/3T3 cells treated with UV-C (50 mJ/cm2), then recovery (1h). ab202272 Recombinant Alexa Fluor® 594 Anti-alpha Tubulin antibody [EP1332Y] - Microtubule Marker (ab202272) was used to counterstain tubulin at 1/50 dilution (10 µg/mL) (Red). The nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150117 Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (2 µg/ml).



Western blot - Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656)

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

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

The expression profile observed is consistent with what has been described in the literature (PMID:11673449 and 11571274).

Exposure time: 3 minutes.

All lanes:
Western blot - Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656) at 1/1000 dilution

Lane 1:
Untreated HeLa (human cervix adenocarcinoma epithelial cell), whole cell lysate at 20 µg

Lane 2:
HeLa treated with 50mJ/cm2 UV-C, then recovery for 30 minutes, whole cell lysate at 20 µg

Lane 3:
Untreated Jurkat (human T cell leukemia T lymphocyte), whole cell lysate at 20 µg

Lane 4:
Jurkat treated with 25uM etoposide for 8 hours, whole cell lysate at 20 µg

Lane 5:
Untreated NIH/3T3 (mouse embryonic fibroblast), whole cell lysate at 20 µg

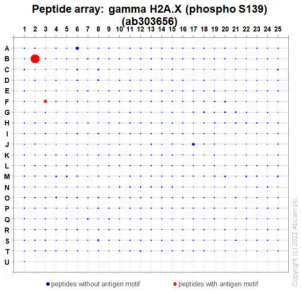
Lane 6:
NIH/3T3 treated with 100mJ/cm2 UV-C, then recovery for 2 hours, whole cell lysate at 20 µg

Secondary

All lanes:
Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/2000 dilution

Observed band size: 15 kDa

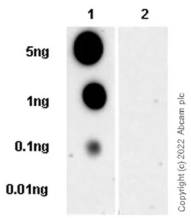
Exposure time: 3min



Peptide Array - Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656)

Peptide array analysis of ab303656 at 0.05 µg/ml followed by a Goat Anti-Rabbit IgG, (H+L), Fluor 647nm conjugated at 1/50,000 dilution. All batches of ab303656 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 the area under the 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.



Dot Blot - Anti-gamma H2A.X (phospho S139) antibody [N1-431] (ab303656)

Gamma H2A.X (phospho S139) Dot Blot staining using mouse Anti-gamma H2A.X (phospho S139) antibody

Dot blot analysis of gamma H2A.X (phospho S139) using ab303656 at 1/1000 dilution followed by a Peroxidase-Conjugated Goat anti-Mouse IgG (H+L) at 1/10,000 dilution. Lane 1: H2A.X (phospho S139) peptide Lane 2: H2A.X non-phospho peptide Exposure time: 3 minutes Blocking and diluting buffer and concentration: 5% NFDM/TBST.

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