


Product datasheet

Anti-gamma H2A.X (phospho S139) antibody ab11174

★★★★★ [27 Abreviews](#) [274 References](#) [8 Images](#)

Overview

| | |
|----------------------------|---|
| Product name | Anti-gamma H2A.X (phospho S139) antibody |
| Description | Rabbit polyclonal to gamma H2A.X (phospho S139) |
| Host species | Rabbit |
| Specificity | Using IF, this antibody was shown to bind to a non-nuclear location in Hela cells. |
| Tested applications | Suitable for: IHC-P, ICC/IF, WB |
| Species reactivity | Reacts with: Mouse, Human Predicted to work with: Rabbit, Guinea pig, Cow, Dog, Pig, Rhesus monkey, Gorilla, Chinese hamster, Bat  |
| Immunogen | Synthetic peptide corresponding to Human gamma H2A.X (phospho S139). Database link: 3014 |
| General notes | <p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p> |

Properties

| | |
|-----------------------------|--|
| Form | Liquid |
| Storage instructions | Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles. |
| Storage buffer | pH: 7 Preservative: 0.1% Sodium azide Constituents: 0.021% PBS, 1.764% Sodium citrate, 1.815% Tris |
| Purity | Immunogen affinity purified |
| Purification notes | Antibodies were affinity purified using the peptide immobilized on solid support. |
| Clonality | Polyclonal |
| Isotype | IgG |

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab11174 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 |
|-------------|------------|---|
| IHC-P | ★★★★★ (5) | 1/1000 - 1/5000. |
| ICC/IF | ★★★★★ (7) | 1/500 - 1/5000. |
| WB | ★★★★★ (11) | 1/2000 - 1/10000. Detects a band of approximately 15 kDa. |

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/P4-kinase family.

Post-translational modifications

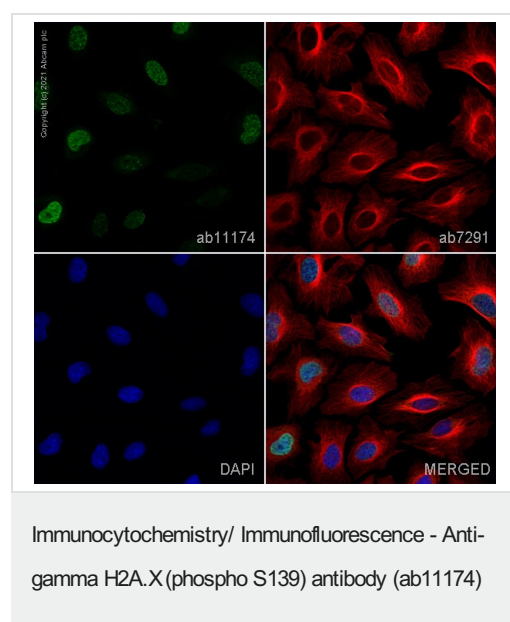
Phosphorylated on Ser-140 (to form gamma-H2AFX or H2AX139ph) in response to DNA double strand breaks (DSBs) generated by exogenous genotoxic agents and by stalled replication forks, and may also occur during meiotic recombination events and immunoglobulin class switching in lymphocytes. Phosphorylation 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 occurs 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

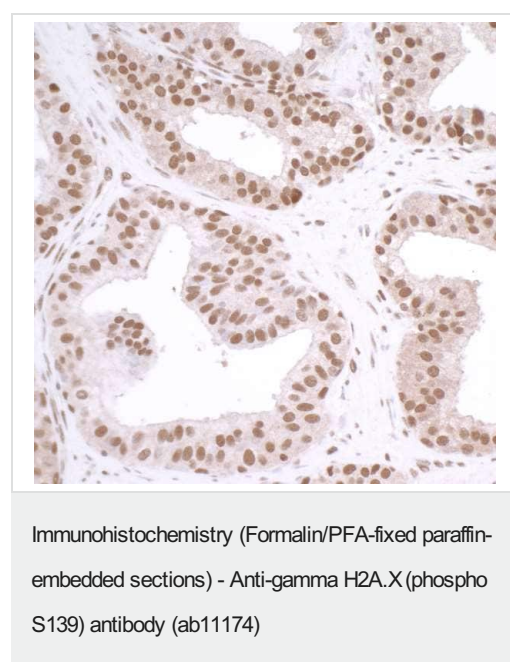
Nucleus. Chromosome.

Images

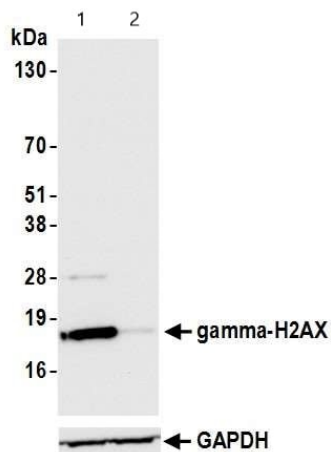


ab11174 staining gamma H2A.X (phospho S139) in HeLa UV cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab11174 at 0.1µg/ml and **ab7291**, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with **ab150081**, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and **ab150120**, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human prostate carcinoma tissue labeling gamma H2A.X (phospho S139) with ab11174 at 1/5000 dilution. Heat mediated antigen retrieval was performed using citrate buffer pH 6.



Western blot - Anti-gamma H2A.X (phospho S139) antibody (ab11174)

All lanes : Anti-gamma H2A.X (phospho S139) antibody (ab11174) at 0.04 μg/ml

Lane 1 : NIH/3T3 (mouse embryo fibroblast cell line) cells treated with 100 μM etoposide, whole cell lysate

Lane 2 : NIH/3T3 cells mock treated, whole cell lysate

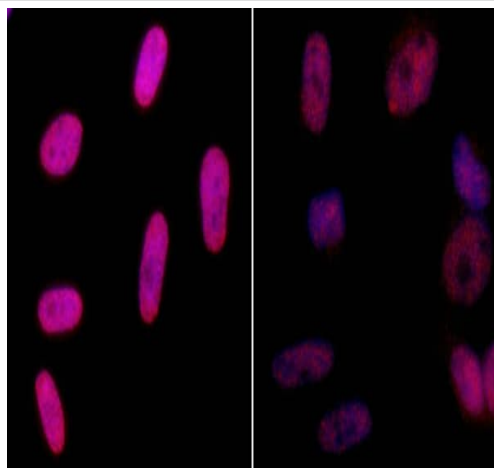
Lysates/proteins at 50 μg per lane.

Secondary

All lanes : Goat anti-rabbit IgG (HRP)

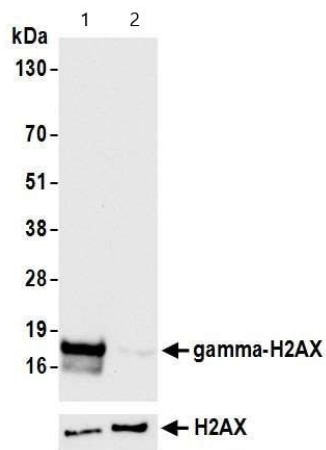
Exposure time: 3 seconds

Lower Panel: Rabbit anti-GAPDH antibody.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab11174)

Immunocytochemistry/Immunofluorescence analysis of neocarzinostatin treated asynchronous HeLa cells (left) and untreated asynchronous HeLa cells (right) labelling H2A.X (phospho S139) with ab11174 at 1/5000 (0.2 μg/ml). A DyLight® 594-conjugated anti-rabbit IgG (1/100) was used as the secondary antibody.



Western blot - Anti-gamma H2A.X(phospho S139) antibody (ab11174)

All lanes : Anti-gamma H2A.X (phospho S139) antibody (ab11174) at 0.04 μg/ml

Lane 1 : Jurkat (human T cell leukemia cell line from peripheral blood) cells treated with 100 μM etoposide, whole cell lysate

Lane 2 : Jurkat cells mock treated, whole cell lysate

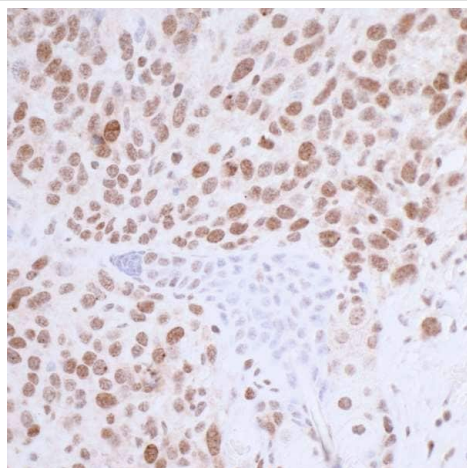
Lysates/proteins at 50 μg per lane.

Secondary

All lanes : Goat anti-rabbit IgG (HRP)

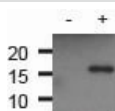
Exposure time: 10 seconds

Lower Panel shows western blot for total H2AX using rabbit anti-H2AX recombinant monoclonal antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X(phospho S139) antibody (ab11174)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse CT26 colon carcinoma tissue labeling gamma H2A.X (phospho S139) with ab11174 at 1/5000 dilution. Heat mediated antigen retrieval was performed using citrate buffer pH 6.



Western blot - Anti-gamma H2A.X(phospho S139) antibody (ab11174)

This image is courtesy of an anonymous Abreview

All lanes : Anti-gamma H2A.X (phospho S139) antibody (ab11174) at 1/5000 dilution

Lane 1 : HeLa nuclear lysate - untreated

Lane 2 : HeLa nuclear lysate - IR treated

Lysates/proteins at 40 μg per lane.

Secondary

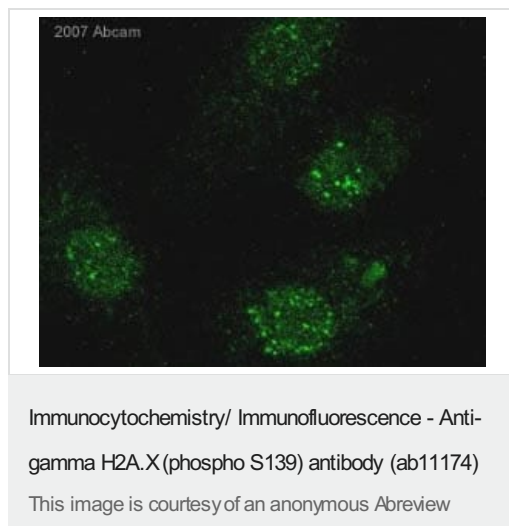
All lanes : HRP-conjugated donkey anti-rabbit IgG polyclonal

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 17 kDa

Exposure time: 30 seconds



ab11174 at 1/1000 staining human HeLa cells by ICC/IF. These cells express a gene that causes a DNA damage response, leading to H2AX phosphorylation. The cells were paraformaldehyde fixed and blocked with BSA prior to incubation with the antibody for 45 minutes. An Alexa-Fluor® 488 conjugated goat anti-rabbit was used as the secondary.

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