abcam

Product datasheet

Anti-gamma H2A.X (phospho S139) antibody [3F2] ab22551

13 Abreviews 150 References 8 Images

Overview

Product name	Anti-gamma H2A.X (phospho S139) antibody [3F2]
Description	Mouse monoclonal [3F2] to gamma H2A.X (phospho S139)
Host species	Mouse
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, Flow Cyt
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide corresponding to Human gamma H2A.X (phospho S139). Synthetic peptide sequence surrounding phosphorylated Ser139
Positive control	WB: Jurkat (treated with staurosporin) cell lysate. ICC: HepG2, A549 and HeLa cells IHC-P: Human breast tissue; Postnatal mouse lung sections with DNA damage in airway cells. Flow Cyt: HeLa cells.
General notes	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. 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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	Preservative: 0.05% Sodium azide Constituents: PBS, 0.1% BSA
Purity	Protein G purified
Clonality	Monoclonal
Clone number	3F2
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab22551 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
WB	★★★★☆ **(4)**	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa.
IHC-P	★★★★☆ **(2)**	Use at an assay dependent concentration.
ICC/IF	★★★★☆ **(7)**	Use a concentration of 2 - 4 µg/ml.
Flow Cyt		Use 1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

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.

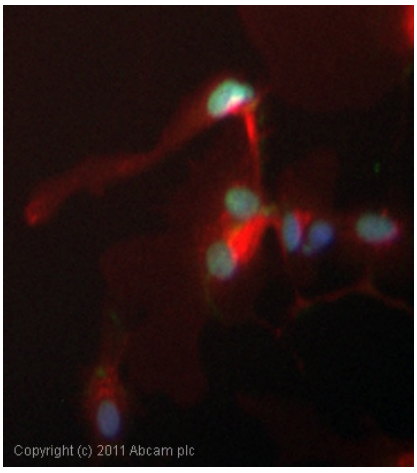
Domain The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.

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 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.

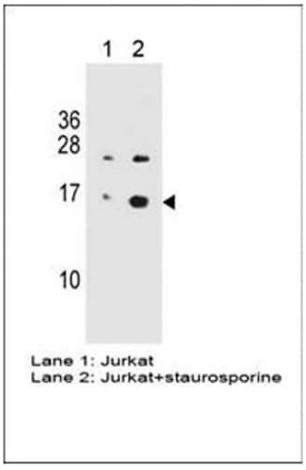
Cellular localization Nucleus. Chromosome.

Images



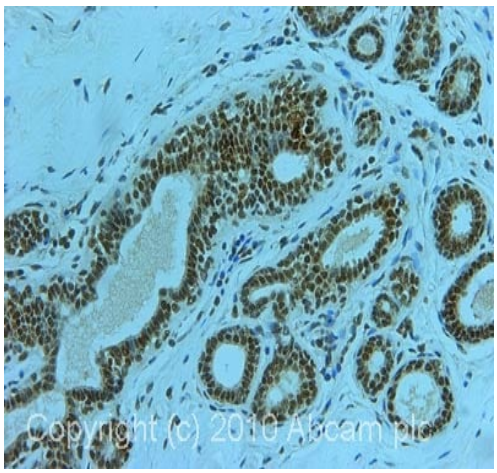
Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [3F2] (ab22551)

ICC/IF image of ab22551 stained HepG2 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab22551, 10µg/ml) overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-mouse IgG - H&L, pre-adsorbed (**ab96879**) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Western blot - Anti-gamma H2A.X (phospho S139) antibody [3F2] (ab22551)

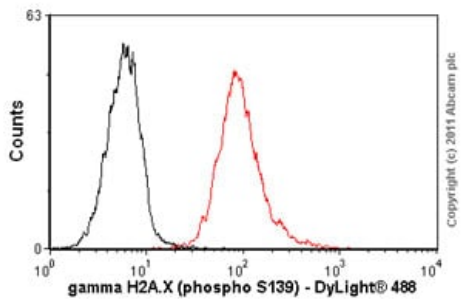
Western blot analysis of Phospho-H2A.X (phospho S139) (ab22551) at a concentration of 1 µg/mL on Jurkat cell untreated (Lane 1) and Jurkat cell stimulated with staurosporine (Lane 2) followed by HRP conjugated goat anti-mouse IgG (H+L) Secondary Antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [3F2] (ab22551)

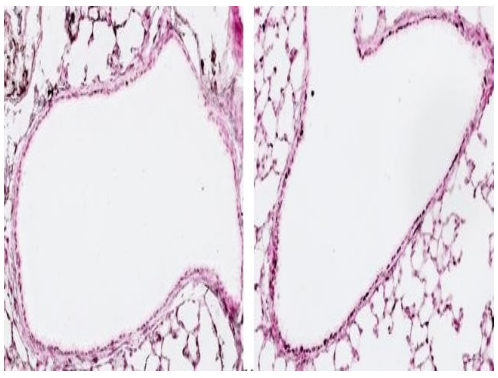
IHC image of ab22551 staining in Human breast formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab22551, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Flow Cytometry - Anti-gamma H2A.X (phospho S139) antibody [3F2] (ab22551)

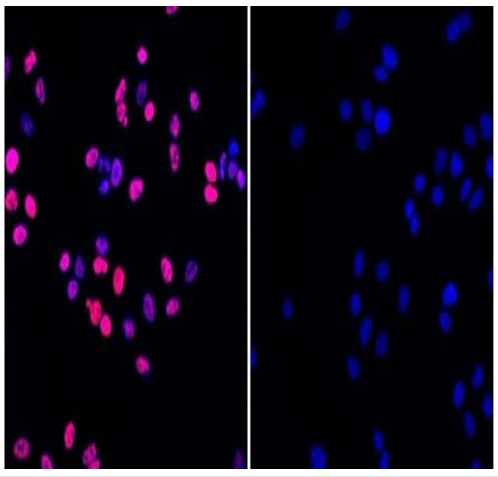
Overlay histogram showing HeLa cells stained with ab22551 (red line). The cells were fixed with 100% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab22551, 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 Mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [3F2] (ab22551)

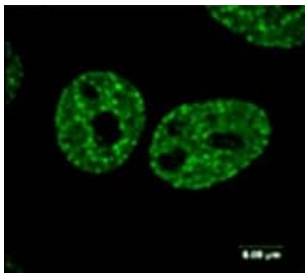
Immunohistochemistry analysis of phospho-Histone H2A.X pSer140 labelled with ab22551 in postnatal mouse (PN19) lung sections. Antigen retrieval from 4% PFA, paraffin embedded sections was performed using heat induced epitope retrieval (HIER) method with sodium citrate buffer (pH 6.0). Following antigen retrieval, tissues were blocked and probed withab22551 at a dilution of 1:400. Increased staining intensity was observed in a genetic mouse model with DNA damage in airway cells.

Left: Control; Right: A genetic model with DNA damage in airway cells



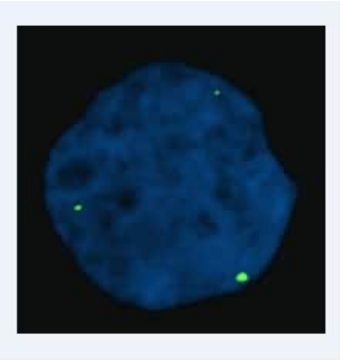
Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [3F2] (ab22551)

Immunofluorescence staining of Phospho-H2AX with ab22551 at 4 ug/ml in A549 cells. Cells were treated with vehicle (control; 0.1% DMSO in media) (right) or with 50 µM etoposide for 1 hour (left).



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [3F2] (ab22551)

ab22551 labelling gamma H2A.X (phospho S139) in HeLa cells by immunocytochemistry/immunofluorescence.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [3F2] (ab22551)

This image is courtesy of Jorge E. Gonzalez (CPHR, La Habana, Cuba), Joan F. Barquinero(UAB, Barcelona,Spain) and Jessica Martinez (UAB, Universitat Autònoma de Barcelona, Spain).

A431 cell nucleus (DAPI signal) showing three gamma-H2AX foci structures.
Primary anitibody H2A.X (phospho S139) antibody [3F2], ab22551, 100ug.
Secondary antibody Mouse IgG-Fc (FITC), **ab97264**, 1mg.

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