

Product datasheet

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

★★★★☆ 15 Abreviews 47 References 7 Images

Overview

Product name	Anti-gamma H2A.X (phospho S139) antibody [9F3]
Description	Mouse monoclonal [9F3] to gamma H2A.X (phospho S139)
Host species	Mouse
Tested applications	Suitable for: Flow Cyt, WB, IP, ICC/IF, IHC-P, In situ hybridization
Species reactivity	Reacts with: Mouse, Rat, Sheep, Rabbit, Chicken, Guinea pig, Hamster, Cow, Dog, Human, Pig, Monkey
Immunogen	Synthetic peptide of phosphorylated (Ser139) human Histone H2A.X.
Positive control	HeLa heat shocked cell lysate for Western blot.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term.
Storage buffer	Preservative: 0.09% Sodium Azide Constituents: 50% Glycerol, PBS, pH 7.2
Purity	Protein G purified
Purification notes	This antibody was purified using affinity chromatography.
Clonality	Monoclonal
Clone number	9F3
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab26350** 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
Flow Cyt		Use 1µg for 10 ⁶ cells.
WB	★★★★☆	Use a concentration of 1 µg/ml. Detects a band of approximately 16 kDa.
IP		Use a concentration of 12.5 µg/ml.
ICC/IF	★★★★☆	1/500.
IHC-P	★★★★★	Use a concentration of 1 µg/ml.
In situ hybridization		Use at an assay dependent dilution.

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

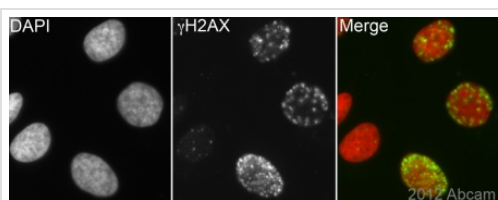
APBB1/FE65 and pro-apoptosis factors such as MAPK8/JNK1, triggering apoptosis. In contrast, dephosphorylation of Tyr-143 by EYA proteins (EYA1, EYA2, EYA3 or EYA4) favors the recruitment of MDC1-containing DNA repair complexes to the tail of phosphorylated Ser-140 (H2AX139ph).

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.

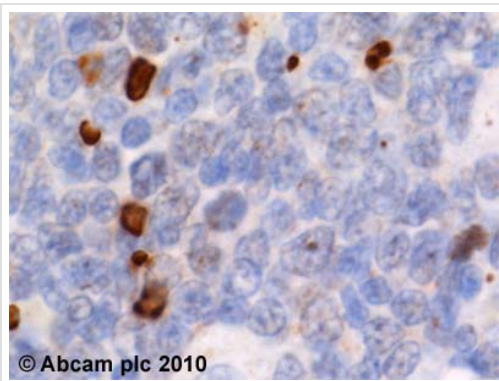
Images



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

Image courtesy of an Abreview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MCB, Canada

ab26350 (1/500) staining gamma H2A.X (phospho S139) in HeLa cells (green). Cells were fixed in paraformaldehyde, permeabilised with 0.5% Triton X-100/PBS and counterstained with DAPI in order to highlight the nucleus (red). For further experimental details please refer to Abreview.

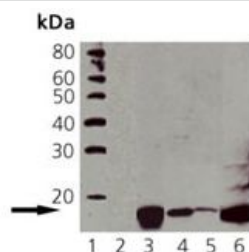


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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

ab26350 (1µg/ml) staining gamma H2A in human spleen, using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear staining .

Sections were rehydrated and antigen retrieved with the Dako 3 in 1 AR buffer citrate pH6.1 in a DAKO PT link. Slides were peroxidase blocked in 3% H₂O₂ in methanol for 10 mins. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 min and detected with Dako envision flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that, for manual staining, optimization of primary antibody concentration and incubation time is recommended. Signal amplification may be required.



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

All lanes : Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

Lane 1 : Molecular weight marker

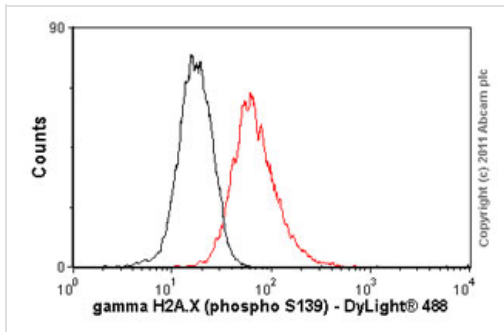
Lane 2 : Cell lysates prepared from human Jurkat cells

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

Lane 4 : Cell lysates prepared from mouse NIH3T3 cells

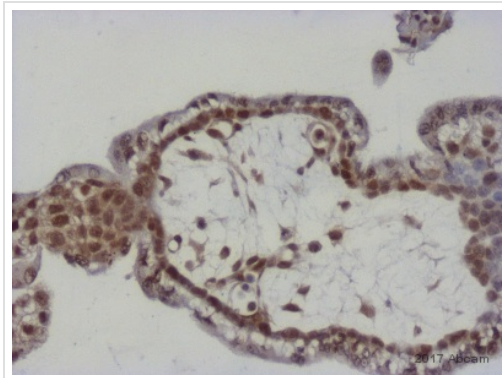
Lane 5 : Cell lysates prepared from CHO-K1 cells

Lane 6 : Cell lysates prepared from Rat-2 cells



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

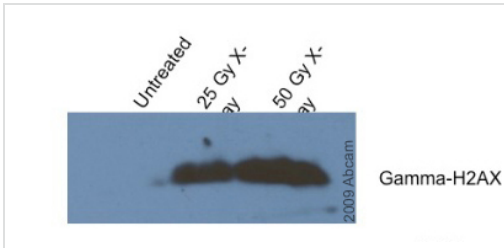
Overlay histogram showing HeLa cells stained with ab26350 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab26350, 1µg/1x10⁶ 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/1x10⁶ cells), IgG2a [ICIGG2A], (ab91361, 1µg/1x10⁶ cells), IgG2b [PLPV219], (ab91366, 1µg/1x10⁶ cells), IgG3 [MG3-35], (ab18394, 1µg/1x10⁶ 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 [9F3] (ab26350)

This image is courtesy of an anonymous Abreview.

Ab26350 staining H2A.X in Human placenta tissue sections by Immunohistochemistry (IHC-P - formaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 5% BSA for 30 minutes at 22°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/100 in TBS) for 16 hours at 4°C. A diluted Biotin conjugated Goat anti-mouse polyclonal (1/200) was used as the secondary antibody.



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

This image is courtesy of an anonymous Abreview

All lanes : Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350) at 1/1000 dilution

Lane 1 : Untreated Zebrafish embryos.

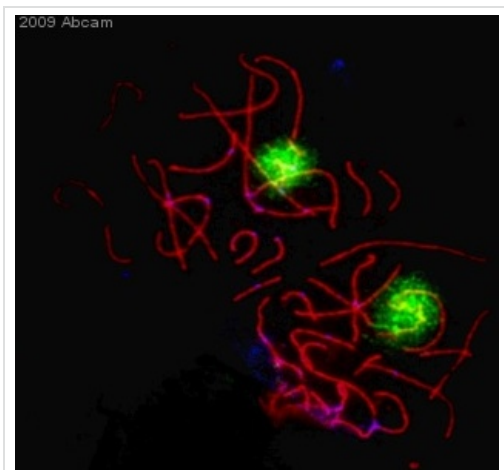
Lane 2 : Zebrafish embryos treated with 25 Gy of X-Ray.

Lane 3 : Zebrafish embryos treated with 50 Gy of X-Ray

Secondary

All lanes : Goat anti-mouse HRP at 1/10000 dilution

Please see abreview for Western blot details.



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

This image is a courtesy of Pinton Alain

ab26350 staining gamma H2AX in pig spermatocytes by Immunocytochemistry/ Immunofluorescence. Cells were fixed with formaldehyde, permeabilized with Triton x100 and blocking with 0.15% BSA was performed for 30 minutes at 25°C was performed. Samples were incubated with primary antibody (1/100: in PBS + 0.15% BSA+0.1% Tween 20) for 12 hours at 25°C. An Alexa Fluor®488-conjugated goat monoclonal to mouse IgG was used at dilution at 1/100 as secondary antibody.

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