

## Product datasheet

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

★★★★☆ 17 Abreviews 64 References 7 Images

### Overview

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

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term.
<b>Storage buffer</b>	Preservative: 0.09% Sodium Azide Constituents: 50% Glycerol, PBS, pH 7.2
<b>Purity</b>	Protein G purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	9F3
<b>Isotype</b>	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 <sup>6</sup> cells.

Application	Abreviews	Notes
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

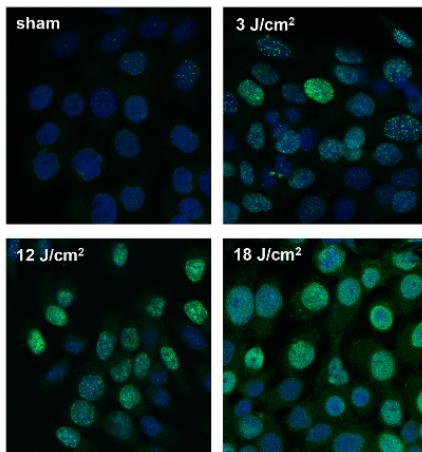
<b>Function</b>	Variant histone H2A which replaces conventional H2A in a subset of nucleosomes. Nucleosomes wrap and compact DNA into chromatin, limiting DNA accessibility to the cellular machineries which require DNA as a template. Histones thereby play a central role in transcription regulation, DNA 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.
<b>Sequence similarities</b>	Belongs to the histone H2A family.
<b>Developmental stage</b>	Synthesized in G1 as well as in S-phase.
<b>Domain</b>	The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.
<b>Post-translational modifications</b>	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



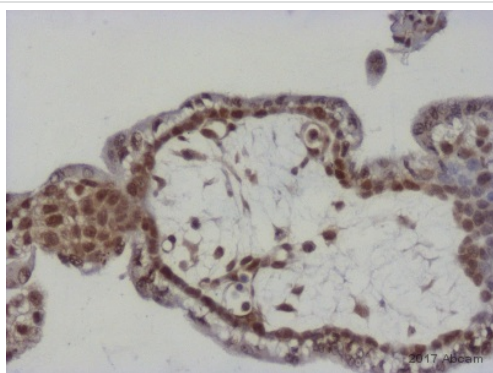
Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody [9F3] (ab26350)

Zhang X et al PLoS One. 2016 Feb 26;11(2):e0150175. doi: 10.1371/journal.pone.0150175. eCollection 2016.

## H2AX phosphorylation and cell viability after ssUV irradiation.

ssUVR induces  $\gamma$ H2AX in human keratinocytes. HaCaT cells were exposed to ssUVR at 0, 3, 12, 18 J/cm<sup>2</sup>, the levels of  $\gamma$ H2AX was determined at 1 hour after irradiation. Representative image.

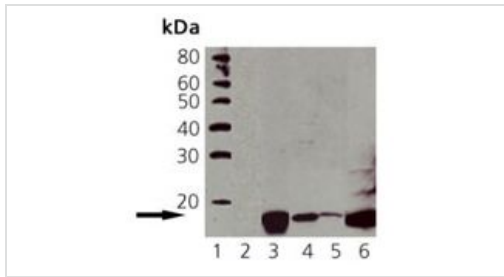
Cells were seed in 6-cm cell culture dishes. On the Next day, cells were exposed to various doses of ssUVR (0, 3, 12, 18 J/cm<sup>2</sup>). At 1 hour after irradiation, cells were fixed with 100% cold methanol at -20°C for 15 minutes, and permeabilized with 0.2% Triton X-100 for 10 minutes. The cells were then blocked with 3% BSA in PBS for 1 hour at room temperature, and incubated overnight at 4°C with  $\gamma$ H2AX monoclonal antibody (Abcam, ab26350). After three washes, cells were incubated with the secondary antibody conjugated with Alexa Fluor 488 (Molecular Probes) for 1 hour at room temperature. The cells were mounted with Prolong Diamond Antifade reagent containing DAPI (Molecular Probes). Images were acquired using Leica SP5 Confocal microscope. The intensity of  $\gamma$ H2AX signals was quantified using ImageJ.



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)

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

**Lane 1 :** Molecular weight marker

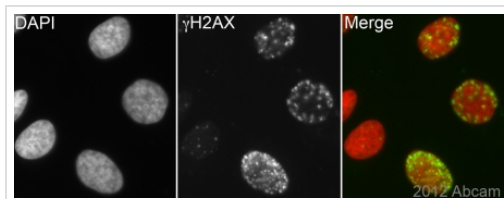
**Lane 2 :** Cell lysates prepared from human Jurkat cells

**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

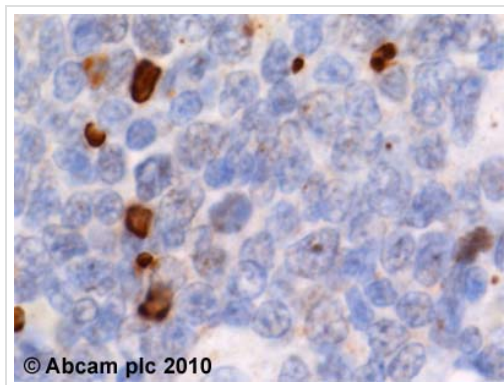
**Lane 6 :** Cell lysates prepared from Rat-2 cells



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 MICB, Canada

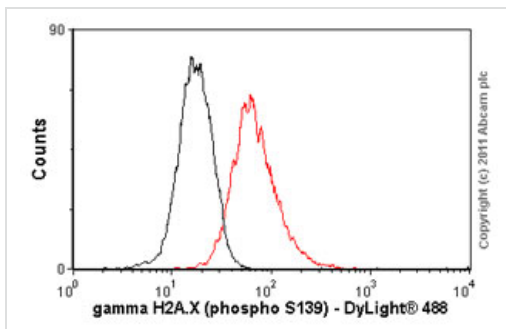
ab26350 (1/500) staining gamma H2A.X (phospho S139) in HeLa cells (green). Cells were fixed in paraformaldehyde, permeabilised with 0.5% Triton X-100/PBS and counterstained with DAPI in order to highlight the nucleus (red). For further experimental details please refer to Abreview.



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

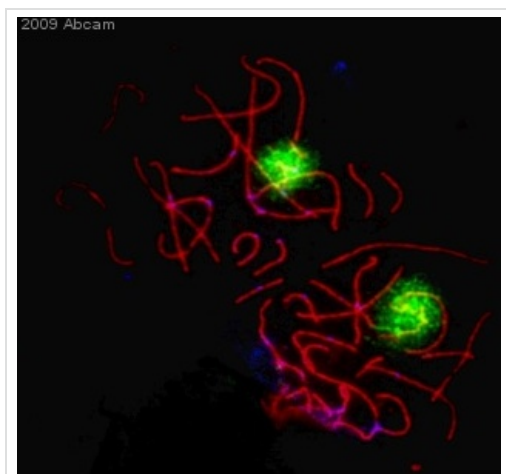
ab26350 (1 µg/ml) staining gamma H2A in human spleen, using an automated system (DAKO Autostainer Plus). Using this protocol there is strong nuclear staining .

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% H<sub>2</sub>O<sub>2</sub> 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.



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/1x10<sup>6</sup> 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<sup>6</sup> cells), IgG2a [ICIGG2A], (ab91361, 1µg/1x10<sup>6</sup> cells), IgG2b [PLPV219], (ab91366, 1µg/1x10<sup>6</sup> cells), IgG3 [MG3-35], (ab18394, 1µg/1x10<sup>6</sup> cells) used under the same conditions. Acquisition of >5,000 events was performed.



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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