Product datasheet

Anti-gamma H2A.X (phospho S139) antibody - ChIP Grade ab2893

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Overview

Product name
Anti-gamma H2A.X (phospho S139) antibody - ChIP Grade

Description
Rabbit polyclonal to gamma H2A.X (phospho S139) - ChIP Grade

Host species
Rabbit

Tested applications
Suitable for: IHC-FoFr, IP, ICC, ICC/IF, IHC-P, ChIP/Chip, ChiP, WB

Species reactivity
Reacts with: Mouse, Rat, Human

Predicted to work with: Chimpanzee

Immunogen
Synthetic peptide conjugated to KLH derived from within residues 100 - 200 of Human gamma H2A.X, phosphorylated at S139. Read Abcam's proprietary immunogen policy (Peptide available as ab15645.)

Positive control
HeLa cell histone preparation and colcemid treated HeLa histone preparation. ICC/IF: Hela cells UV treated.

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 or -80°C. Avoid freeze / thaw cycle.

Storage buffer
Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4

Purity
Immunogen affinity purified

Clonality
Polyclonal

Isotype
IgG

Applications

Our Abpromise guarantee covers the use of ab2893 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
**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 occur at unsynapsed regions of both autosomes and the XY bivalent during zygote, 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

<table>
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<tr>
<td>IHC-FoFr</td>
<td></td>
<td>1/200.</td>
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<tr>
<td>IP</td>
<td><img src="2/5" alt="star" /> <img src="2/5" alt="star" /> <img src="2/5" alt="star" /> <img src="2/5" alt="star" /> <img src="2/5" alt="star" /></td>
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<td>1/400.</td>
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<td>Use at an assay dependent concentration.</td>
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<td>1/50.</td>
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<td>WB</td>
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<td>1/1000. Detects a band of approximately 17 kDa (predicted molecular weight: 17 kDa).</td>
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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**

ab2893 used at 1/100 dilution in ChIP.
Nuclear lysate prepared from HEK-293 (human epithelial cell line from embryonic kidney) cells. Cross-linking (X-ChIP) 15 minutes. Detection step: Real-time PCR.
Western blot - Anti-gamma H2A.X (phospho S139) antibody - ChIP Grade (ab2893)

Rabbit polyclonal to H2A.X (phospho S139) - ab2893 used at 1/500.

Blocking peptides at 1ug/lane.

Lanes 1, 3 and 5: Control HeLa cell histone preparation
Lanes 2, 4 and 6: Colcemid treated HeLa histone preparation

Lanes 1 and 2: ab2893
Lanes 3 and 4: ab2893 + Histone H2A.X (phospho Ser 139) peptide - ab15645
Lanes 5 and 6: ab2893 + Histone H2A.X non-phospho peptide - ab15646

Secondary antibody - ab6721 - Goat polyclonal to Rabbit IgG (HRP) at 1/5000.

Rabbit polyclonal to H2A.X (phospho S139) - ab2893 used at 1/500.

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Secondary antibody - ab6721 - Goat
ICC/IF image of ab2893 stained UV treated HeLa cells. The cells were 100% methanol fixed (5 min) then permeabilised using 0.1% PBS-Triton and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to further permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab2893 at 1µg/ml overnight at +4°C. The secondary antibody (pseudo-colored green) was Alexa Fluor® 488 goat anti- rabbit (ab150081) IgG (H+L) preadsorbed, used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1h at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.
ab2893 staining gamma H2A.X in human mammary tissue by Immunohistochemistry (paraffin embedded sections).

Paraffin-embedded blocks were sectioned and mounted on frost-free slides. The 3-10 µm sections were deparaffinized in xylene and rehydrated through a series of graded alcohols. Slides were washed with 1× PBS and endogenous peroxidases were blocked with 1.5% hydrogen peroxide in 1× PBS for 20 minutes at 25°C. After three 5 minutes washes in 1× PBS, slides were incubated in blocking solution (1× PBS with 0.1% Triton X-100, 3% bovine serum albumin) with 5% normal donkey serum for 10 minutes at 25°C. Control (no primary antibody) and experimental slides were incubated overnight at 4°C, respectively, in blocking solution alone or blocking solution with ab2893 at 1/1000 dilution. Biotin-conjugated secondary antibody 1/200 was added and slides were incubated at 25°C for 30 minutes and then washed three times with 1× PBS. The ABC Peroxidase Staining kit (1/100 dilution of each Rea

ab2893 used in ChIP.

Nuclear lysate prepared from murine testis cells (irradiated with 20 Gy).

Cross-linking (X-ChIP) 15 minutes.

Detection step: Real-time PCR.

ab2893 used at a 1/50 dilution.
Asynchronous HeLa cells were paraformaldehyde fixed and immunofluorescently labeled with ab2893 that had been preincubated with either 1) non-phosphorylated or 2) phosphorylated H2AX peptide. Identical exposure times were employed. The Merge images present the DAPI and ab2893 channels as red and green, respectively. Scale bars represent 5µm.

1) Non-phosphorylated peptides

2) Phosphorylated peptides

HeLa cells were incubated at 37°C for 3h with vehicle control (0 µM) and different concentrations of camptothecin (ab120115). Increased expression of γH2A.X (phospho S139) in HeLa cells correlates with an increase in camptothecin concentration, as described in literature.

Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 20µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab2893 at 1 µg/ml and ab10475 at 1 µg/ml overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP (ab97051) at 1/10000 dilution and visualised using ECL development solution.
ab2893 staining γH2A.X in MALME-3M cells treated with terfenadine (ab120270), by ICC/IF. Increase of γH2A.X nuclear expression correlates with increased concentration of terfenadine, as described in literature. The cells were incubated at 37°C for 6 hours in media containing different concentrations of ab120270 (terfenadine) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab2893 (10 μg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

ab2893 staining γH2AX (phospho S139) in HeLa cells treated with camptothecin (ab120115), by ICC/IF. Increased nuclear expression of γH2AX (phospho S139) correlates with increased concentration of camptothecin, as described in literature. The cells were incubated at 37°C for 3h in media containing different concentrations of ab120115 (camptothecin) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab2893 (10 μg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 goat anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.
ab2893 staining γH2A.X in HeLa cells treated with SN 38 (ab141108), by ICC/IF. Increase of γH2A.X nuclear expression correlates with increased concentration of SN 38, as described in literature.

The cells were incubated at 37°C for 6 hours in media containing different concentrations of ab141108 (SN 38) in DMSO, fixed with 100% methanol for 5 minutes at -20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab2893 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

ab2893 staining γH2A.X in HeLa cells treated with CPT 11 (Irinotecan) (ab141107), by ICC/IF. Increase of γH2A.X nuclear expression correlates with increased concentration of CPT 11 (Irinotecan), as described in literature.

The cells were incubated at 37°C for 6 hours in media containing different concentrations of ab141107 (CPT 11 (Irinotecan)) in DMSO, fixed with 100% methanol for 5 minutes at 20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab2893 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.
ab2893 staining γH2A.X in HeLa cells treated with 10-Hydroxy camptothecin (ab141071), by ICC/IF. Increase of γH2A.X nuclear expression correlates with increased concentration of 10-Hydroxy camptothecin, as described in literature. The cells were incubated at 37°C for 6 hours in media containing different concentrations of ab141071 (10-Hydroxy camptothecin) in DMSO, fixed with 100% methanol for 5 minutes at -20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab2893 (5 μg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

ab2893 staining γH2A.X in weri cells treated with TMPyP4 tosylate (ab120793), by ICC/IF. Increase of γH2A.X nuclear expression correlates with increased concentration of TMPyP4 tosylate, as described in literature. The cells were incubated at 37°C for 24 hours in media containing different concentrations of ab120793 (TMPyP4 tosylate) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab2893 (1 μg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (ab96899) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.
Asynchronous HeLa cells were exposed to 2Gy and permitted to recover for 30min. Cells were paraformaldehyde fixed (4%), immunofluorescently labeled with ab2893 and counterstained with DAPI. The merge image presents the DAPI and ab2893 channels as red and green, respectively. The scale bar represents 5µm.

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