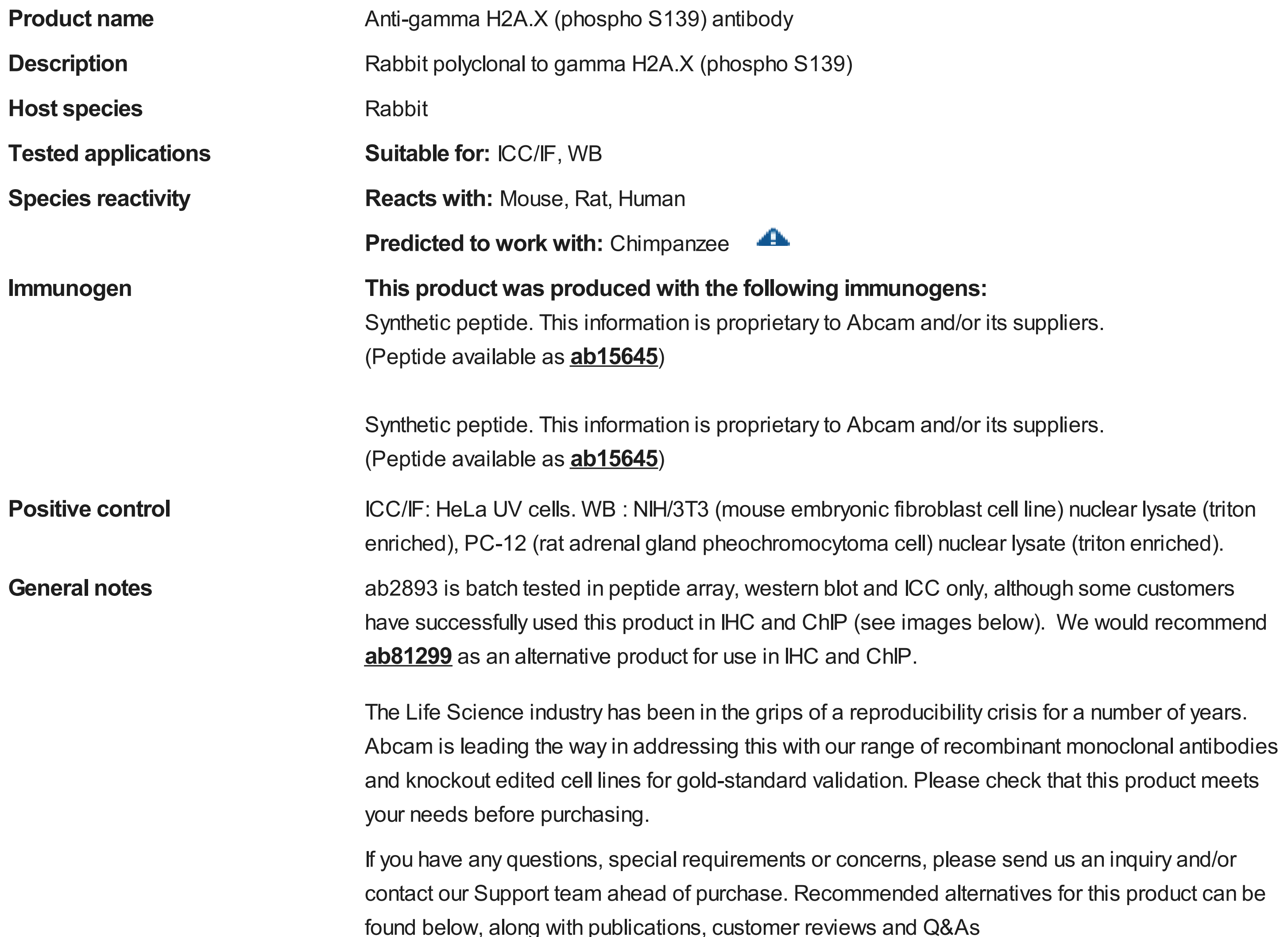
abcam

Product datasheet

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

★★★★☆ 36 Abreviews 366 References 14 Images

Overview



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

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

Host species Rabbit

Tested applications **Suitable for:** ICC/IF, WB

Species reactivity **Reacts with:** Mouse, Rat, Human

Predicted to work with: Chimpanzee

Immunogen **This product was produced with the following immunogens:**
Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
(Peptide available as **ab15645**)

Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
(Peptide available as **ab15645**)

Positive control ICC/IF: HeLa UV cells. WB : NIH/3T3 (mouse embryonic fibroblast cell line) nuclear lysate (triton enriched), PC-12 (rat adrenal gland pheochromocytoma cell) nuclear lysate (triton enriched).

General notes ab2893 is batch tested in peptide array, western blot and ICC only, although some customers have successfully used this product in IHC and ChIP (see images below). We would recommend **ab81299** as an alternative product for use in IHC and ChIP.

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. 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 pH: 7.40

Preservative: 0.02% Sodium azide
Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee 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.

Application	Abreviews	Notes
ICC/IF	★★★☆☆ (7)	Use a concentration of 0.1 µg/ml.
WB	★★★★★ (11)	Use a concentration of 1 µg/ml. Detects a band of approximately 17 kDa (predicted molecular weight: 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.

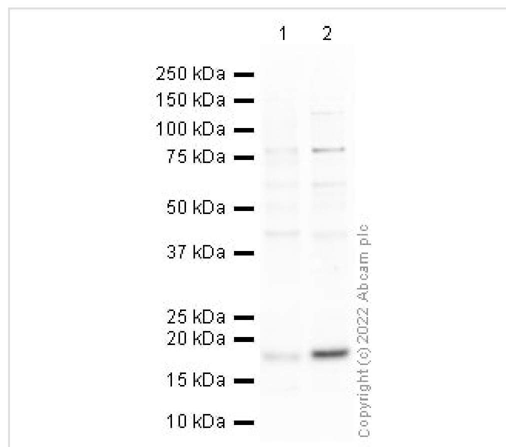
Domain The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.

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



Western blot - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

All lanes : Anti-gamma H2A.X (phospho S139) antibody (ab2893) at 1 µg/ml

Lane 1 : NIH/3T3 (mouse embryonic fibroblast cell line) nuclear lysate (triton enriched)
Lane 2 : PC-12 (rat adrenal gland pheochromocytoma cell) nuclear lysate (triton enriched)

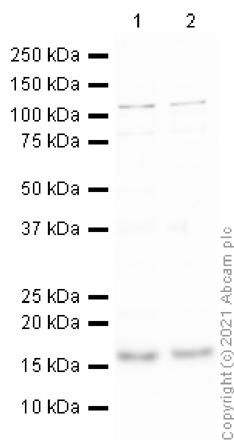
Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

Predicted band size: 15 kDa
Observed band size: 17 kDa

Exposure time: 16 minutes

Gel type : MES
Blocking buffer : 2% BSA block



Western blot - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

All lanes : Anti-gamma H2A.X (phospho S139) antibody (ab2893) at 1 µg/ml

Lane 1 : NIH 3T3 nuclear lysate (triton enriched)

Lane 2 : PC12 nuclear lysate (triton enriched)

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat polyclonal to Rabbit IgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

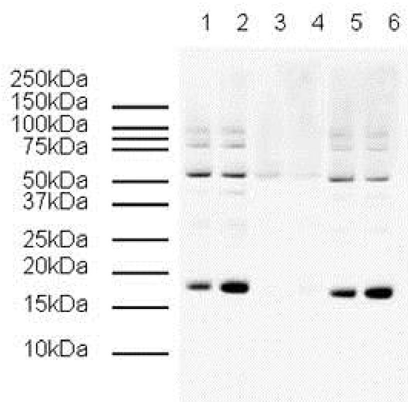
Predicted band size: 15 kDa

Observed band size: 17 kDa

Exposure time: 4 minutes

Gel type: MES

Blocking buffer: 2% BSA block



Western blot - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

All lanes : Anti-gamma H2A.X (phospho S139) antibody (ab2893) at 1/500 dilution

Lanes 1 & 3 & 5 : Control HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate Histone preparation

Lane 2 : Colcemid treated HeLa whole cell lysate Histone preparation

Lanes 4 & 6 : Colcemid treated HeLa whole cell lysate Histone preparation

Secondary

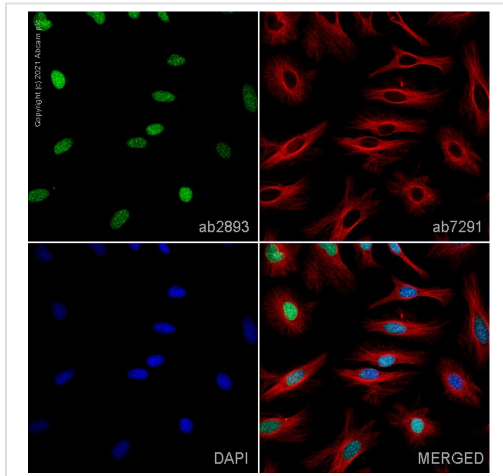
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab6721**) at 1/5000 dilution

Predicted band size: 15 kDa

Observed band size: 17 kDa

Additional bands at: 50 kDa (possible cross reactivity)

Blocking peptides at 1ug/lane.

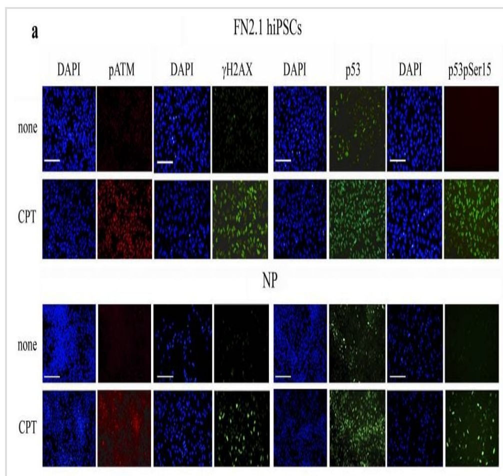


Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

ab2893 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 ab2893 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).

Also suitable in cells fixed with 4% paraformaldehyde (10 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

Garcia, C.P. et al PLoS One. 2016; 11(3): e0152607. Fig.2a Published online 2016 Mar 31. doi: 10.1371/journal.pone.0152607 Reproduced under the Creative Commons licence https://creativecommons.org/licenses/by/4.0/

Immunofluorescence photomicrographs of genotoxic-treated (1µM during 3 h) human induced pluripotent stem cells (hiPSCs) and hESCs-derived neuroprogenitors (NP) performed immediately after CPT treatment (1µM during 3 h). The figure shows representative images of cells stained with primary antibodies against ATM phospho-serine1981 (pATM), histoneγH2AX, p53, p53 phospho serine 15 (p53pSer15). Nuclei were counterstained with DAPI. The scale bars represent 100 µm.

Histone gamma H2A.X was detected using ab2893.

From Figure 2a of Garcia et al **PLoS One**. 2016; 11(3): e0152607. Published online 2016 Mar 31. doi: **10.1371/journal.pone.0152607**

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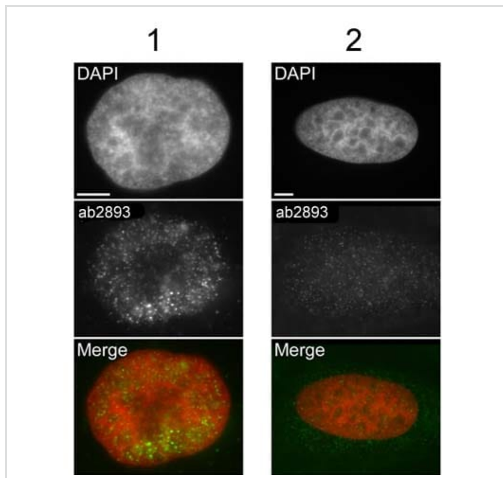


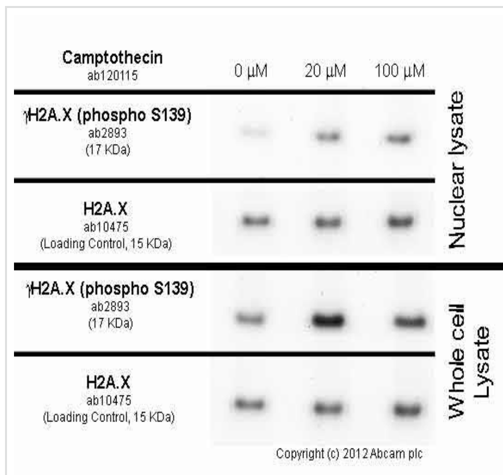
Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

This image is courtesy of Kirk McManus

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

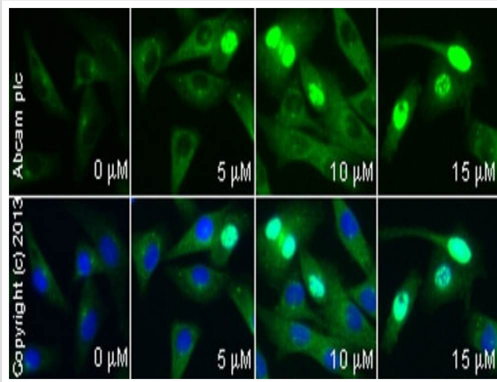
2) Phosphorylated peptides



Western blot - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

HeLa (Human epithelial cell line from cervix adenocarcinoma) 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.

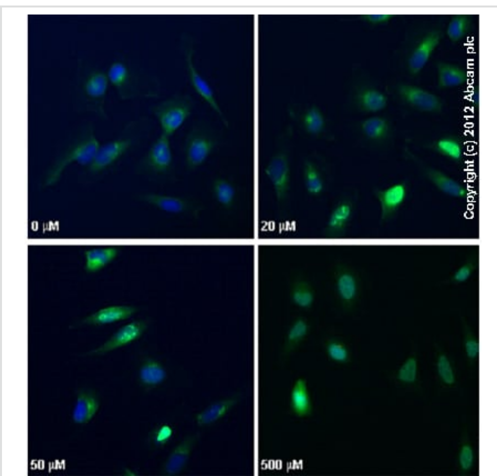
Whole cell lysates were prepared with RIPA buffer (containing protease inhibitors and sodium orthovanadate), 20µg of each were loaded on the gel and the WB was run under reducing conditions. After transfer the membrane was blocked for an hour using 5% BSA before being incubated with ab2893 at 1 µg/ml 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.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

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.

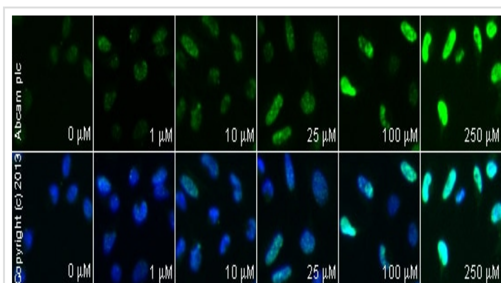
The cells were incubated at 37°C for 6 hours in media containing different concentrations of **ab120270** (terfenadine) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. 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.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

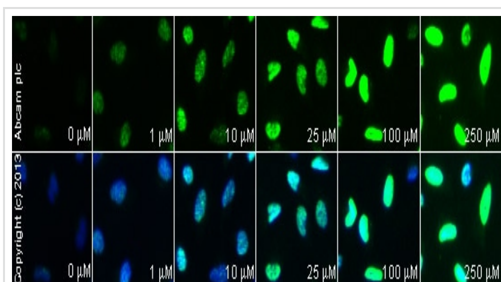
ab2893 staining γH2AX (phospho S139) in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with camptothecin (**ab120115**), by ICC/IF. Increased nuclear expression of γH2AX (phospho S139) correlates with increased concentration of camptothecin, as described in literature.

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.



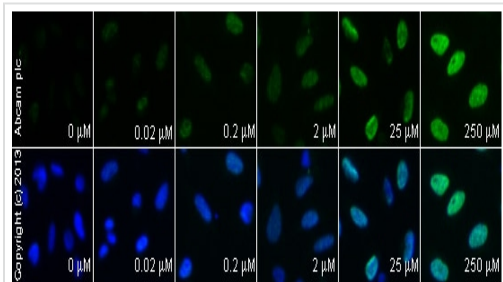
Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

ab2893 staining γH2A.X in HeLa (Human epithelial cell line from cervix adenocarcinoma) 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.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

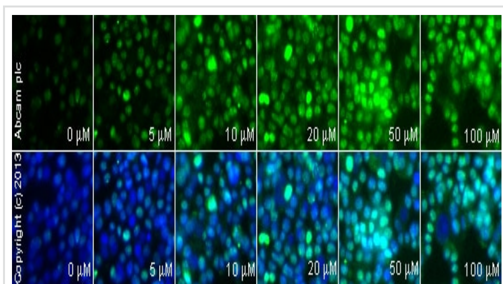
ab2893 staining γH2A.X in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with CPT 11 (Irinotecan) (**ab141107**), by ICC/IF. Increase of γH2A.X nuclear expression correlates with increased concentration of CPT 11 (Irinotecan), as described in literature.
The cells were incubated 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.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

ab2893 staining γH2A.X in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells treated with 10-Hydroxycamptothecin (**ab141071**), by ICC/IF. Increase of γH2A.X nuclear expression correlates with increased concentration of 10-Hydroxycamptothecin, as described in literature.

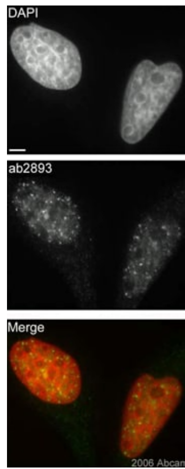
The cells were incubated at 37°C for 6 hours in media containing different concentrations of **ab141071** (10-Hydroxycamptothecin) in DMSO, fixed with 100% methanol for 5 minutes at -20°C and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. 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.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)

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.



Immunocytochemistry/ Immunofluorescence - Anti-gamma H2A.X (phospho S139) antibody (ab2893)
Image courtesy of Dr. Kirk McManus

Asynchronous HeLa (Human epithelial cell line from cervix adenocarcinoma) 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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