abcam

Product datasheet

Anti-Histone H2A.X antibody ab10475

KO VALIDATED

5 References 4 Images

Overview

Product name	Anti-Histone H2A.X antibody
Description	Rabbit polyclonal to Histone H2A.X
Host species	Rabbit
Tested applications	**Suitable for:** WB, ELISA, IHC-P
Species reactivity	**Reacts with:** Cow, Human
Immunogen	Synthetic peptide corresponding to Human Histone H2A.X aa 100 to the C-terminus (C terminal) conjugated to Keyhole Limpet Haemocyanin (KLH). Synthetic peptide derived from residues 100 - 200 of Human H2A.X. Read Abcam's proprietary immunogen policy. Database link: P16104 (Peptide available as ab15020)
Positive control	Calf Thymus Histone Preparation

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

Our Abpromise guarantee covers the use of **ab10475** 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		1/500 - 1/1000. Detects a band of approximately 15 kDa (predicted molecular weight: 15 kDa).
ELISA		1/100000.
IHC-P		Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

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.

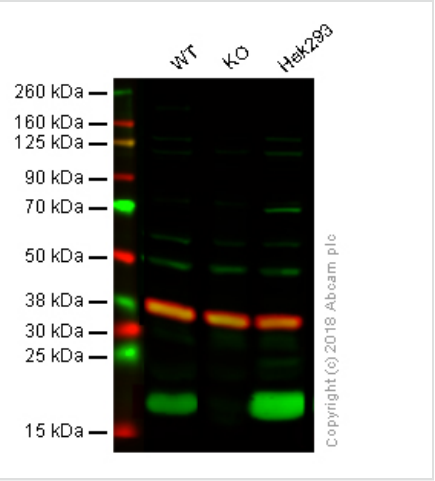
Post-translational modifications

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-Histone H2A.X antibody (ab10475)

All lanes : Anti-Histone H2A.X antibody (ab10475) at 1/500 dilution

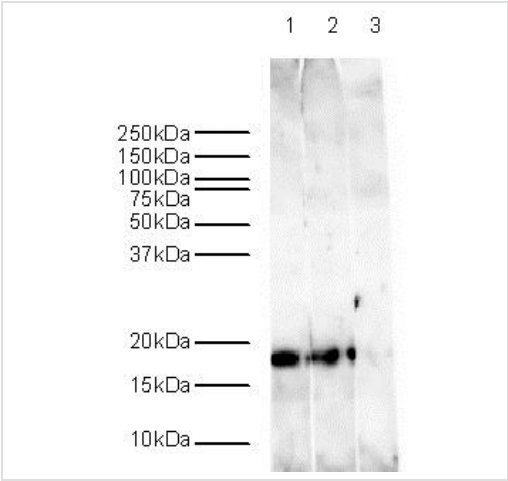
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : H2AFX (Histone H2A.X) knockout HAP1 whole cell lysate
Lane 3 : HEK293 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 15 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab10475 observed at 17 kDa. Red - loading control, ab9484, observed at 37 kDa.

ab10475 was shown to specifically react with Histone H2A.X in wild-type HAP1 cells as signal was lost in H2AFX (Histone H2A.X) knockout cells. Wild-type and H2AFX (Histone H2A.X) knockout samples were subjected to SDS-PAGE. Ab10475 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Histone H2A.X antibody (ab10475)

WB using ab10475

Lanes 1 & 3 : Anti-Histone H2A.X antibody (ab10475) at 1/500 dilution

Lane 2 : Anti-Histone H2A.X antibody (ab10475) at 1/1000 dilution

Lanes 1-2 : Calf thymus histone lysate

Lane 3 : Calf thymus histone lysate with Human Histone H2A.X (unmodified) peptide (ab15020) at 1 µg

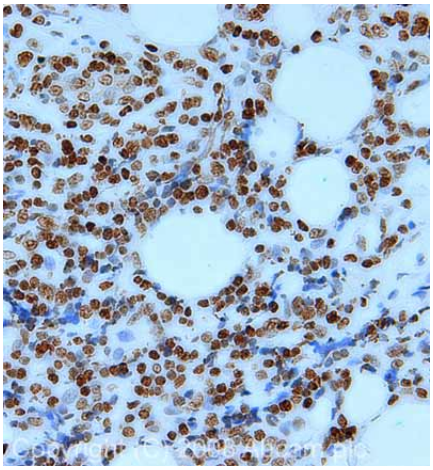
Secondary

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

Performed under reducing conditions.

Predicted band size: 15 kDa

Exposure time: 30 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody (ab10475)

IHC image of Histone H2A X staining in human B cell lymphoma FFPE section, performed on a 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 ab10475, 1µg/ml, for 8 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.

Western blot - Anti-Histone H2A.X antibody (ab10475)

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.

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