abcam

Product datasheet

Anti-Histone H2A.X antibody - ChIP Grade ab11175

★★★★★ 36 Abreviews 80 References 5 Images

Overview

Product name	Anti-Histone H2A.X antibody - ChIP Grade
Description	Rabbit polyclonal to Histone H2A.X - ChIP Grade
Tested applications	**Suitable for:** IHC-P, WB, IP, ChIP, ICC/IF, IHC-Fr
Species reactivity	**Reacts with:** Mouse, Rat, Human, Monkey **Predicted to work with:** Rabbit, Rhesus monkey, Gorilla
Immunogen	This information is considered to be commercially sensitive.
Positive control	Tested with human HEK293, human G-361 and mouse embryonic fibroblast cells.
General notes	The phosphorylated form of this Ab is known as gamma H2A.X, when phosphorylated at Ser 139.

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. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.1% Sodium azide Constituent: Tris citrate/phosphate
Purity	Immunogen affinity purified
Purification notes	Antibodies were affinity purified using the peptide immobilized on solid support.
Clonality	Polyclonal
Isotype	IgG

Applications

Our Abpromise guarantee covers the use of **ab11175** 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
IHC-P	★★★★★	Use at an assay dependent concentration.

Application	Abreviews	Notes
WB	★★★★★	1/5000 - 1/15000. Detects a band of approximately 15 kDa.
IP	★★★★★	Use at 5-20 µg/mg of lysate.
ChIP		Use 2 µg for 25 µg of chromatin. PubMed: 19380460
ICC/IF	★★★★☆	1/2500. (see review). Use periodate-lysine-PFA fixative.
IHC-Fr	★★★★★	1/1000.

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.

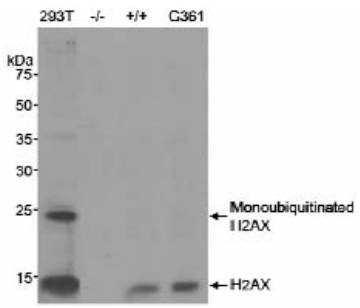
Post-translational modifications

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.

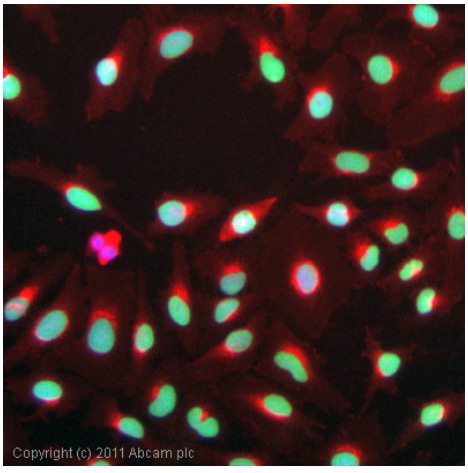
Images



Western blot - Anti-Histone H2A.X antibody - ChIP Grade (ab11175)

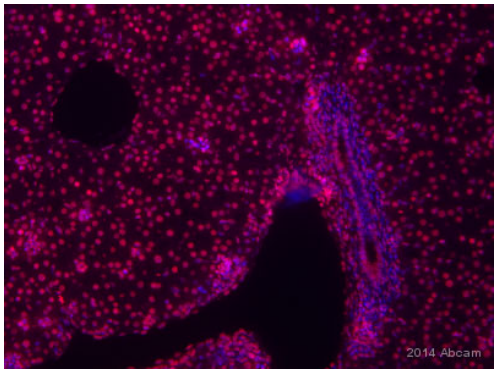
Samples: Nuclear extract from human 293T, human G-361, and wild-type (+/+) or H2AX knockout (-/-) mouse embryonic fibroblasts. *Antibody:* ab11175 used at 0.1 mcg/ml. *Detection:* Chemiluminescence with an exposure time of 5 seconds.

Samples: Nuclear extract from human 293T, human G-361, and wild-type (+/+) or H2AX knockout (-/-) mouse embryonic fibroblasts. Antibody: ab11175 used at 0.1 mcg/ml. Detection: Chemiluminescence with an exposure time of 5 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-Histone H2A.X antibody - ChIP Grade (ab11175)

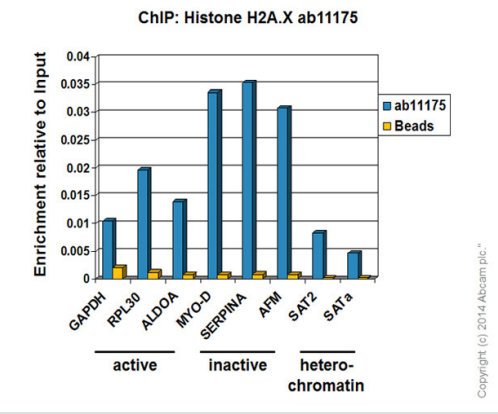
ICC/IF image of ab11175 stained Hela cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab11175, 1µg/ml) overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti-rabbit IgG - H&L, pre-adsorbed (ab96899) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody - ChIP Grade (ab11175)

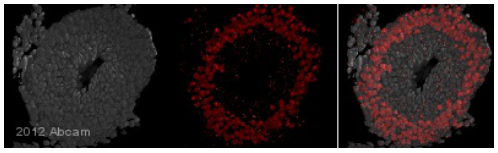
This image is courtesy of an anonymous Abreview.

ab11175 staining Histone H2A.X in mouse liver tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde and blocked with 5% BSA for 60 minutes at 25°C; antigen retrieval was by heat mediation in a Na-citrate pH6 buffer. Samples were incubated with primary antibody (1/100 in PBS/Triton) for 3 hours at 25°C. A Alexa Flour® 555-conjugated donkey anti-rabbit IgG polyclonal (1/250) was used as the secondary antibody.



ChIP - Anti-Histone H2A.X antibody - ChIP Grade (ab11175)

Chromatin was prepared from HeLa cells according to the Abcam X-ChIP protocol. Cells were fixed with formaldehyde for 10 minutes. The ChIP was performed with 25µg of chromatin, 2µg of ab11175 (blue), and 20µl of Protein A/G sepharose beads. No antibody was added to the beads control (yellow). The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci). Primers and probes are located in the first kb of the transcribed region.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Histone H2A.X antibody - ChIP Grade (ab11175)

This image is courtesy of an anonymous Abreview

ab11175 staining Histone H2A.X in Mouse testis tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100 and blocked with 4% BSA for 2 hours at 37°C; antigen retrieval was by heat mediation in a citrate buffer. Samples were incubated with primary antibody (1/100 in diluent) for 48 hours at 4°C. ab6564 Goat polyclonal anti-Rabbit IgG - H&L Cy5® (1/300) was used as the secondary antibody.

Staining left to right:
1) DAPI;
2) Histone H2A.X;
3) Merge

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit http://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors