abcam

Product datasheet

Anti-gamma H2A.X (phospho S139) antibody [BLR053F] ab243906

Recombinant

2 References 7 Images

Overview

Product name	Anti-gamma H2A.X (phospho S139) antibody [BLR053F]
Description	Rabbit monoclonal [BLR053F] to gamma H2A.X (phospho S139)
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC, IHC-P, IP
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Synthetic peptide corresponding to Human gamma H2A.X (phospho S139). Surrounding Serine 139. NP_002096.1 and Gene ID 3014. Database link: **P16104**
Positive control	IP: Jurkat cells; IHC: human basal cell carcinoma, mouse CT26 colon carcinoma, human breast carcinoma; ICC: HeLa cells; WB: Jurkat cells.
General notes	This product is sold under License from Bethyl Laboratories, Inc.

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.8 Preservative: 0.09% Sodium azide Constituents: 98% Borate buffered saline, 0.1% BSA
Purification notes	Recombinant antibody was purified from cell culture supernatant.
Clonality	Monoclonal
Clone number	BLR053F
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab243906 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		1/1000.
ICC		1/100 - 1/500.
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration. Use 20µl/mg lysate.

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.

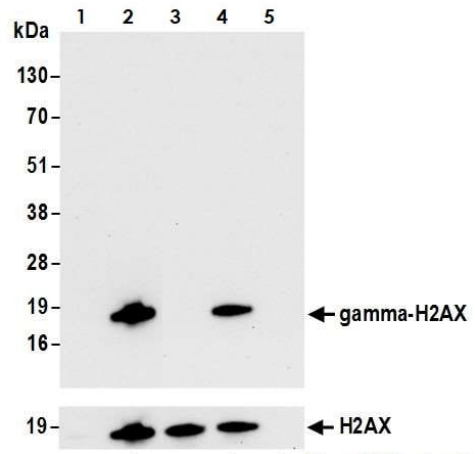
Domain The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.

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



Immunoprecipitation - Anti-gamma H2A.X (phospho S139) antibody [BLR053F] (ab243906)

Gamma H2A.X was immunoprecipitated from 1.0 mg Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate with ab243906 at 20 µl per reaction. Western blot was performed on the immunoprecipitate using **ab243864** at 1/1000 dilution.

Lane 1: ab243906 IP in Jurkat (untreated) whole cell lysate.

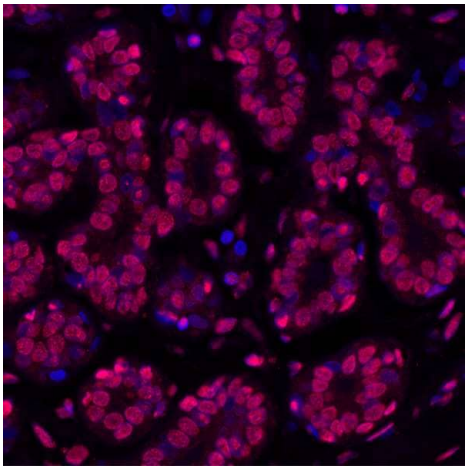
Lane 2: ab243906 IP in Jurkat (treated with Etoposide) whole cell lysate.

Lane 3: rabbit anti-gamma-H2AX antibody IP in Jurkat (untreated) whole cell lysate.

Lane 4: rabbit anti-gamma-H2AX antibody IP in Jurkat (treated with Etoposide) whole cell lysate

Lane 5: Control IgG IP in Jurkat (treated with Etoposide) whole cell lysate.

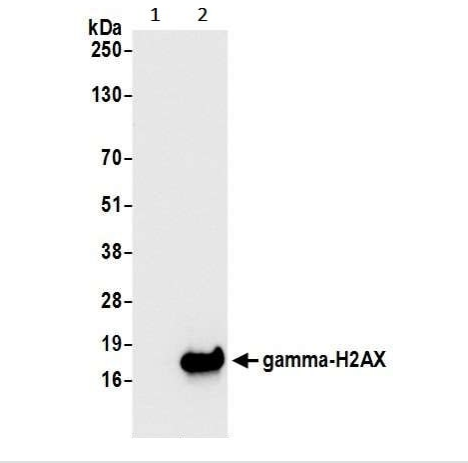
Detection: Chemiluminescence with an exposure time of 3 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [BLR053F] (ab243906)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labelling gamma H2A.X with **ab2439064** at 1/100 dilution, and secondary DyLight® 594 conjugated goat anti-rabbit IgG. Counterstain was DAPI.

Heat mediated antigen retrieval performed with citrate buffer pH 6 before commencing with IHC staining protocol.



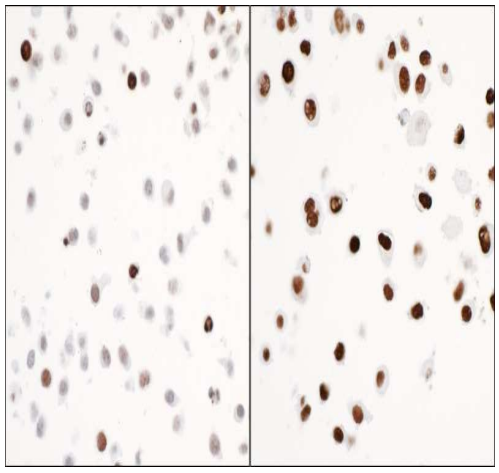
Western blot - Anti-gamma H2A.X (phospho S139) antibody [BLR053F] (ab243906)

Western blot analysis using ab243906 at 1/1000 dilution.

Lane 1: Jurkat (human T cell leukemia cell line from peripheral blood) (untreated) whole cell lysate (50 µg) .

Lane 2: Jurkat (Etoposide-treated) whole cell lysate (50 µg).

A HRP-conjugated goat anti-rabbit IgG antibody was used as the secondary.

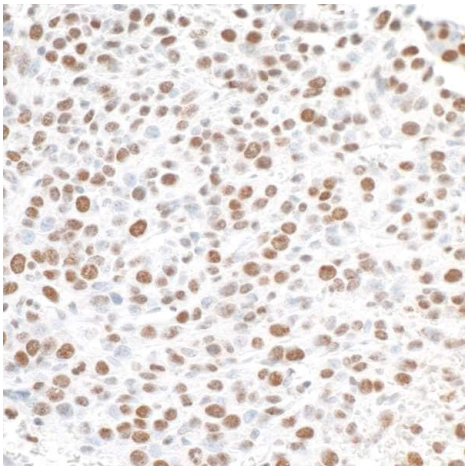


Immunocytochemistry - Anti-gamma H2A.X (phospho S139) antibody [BLR053F] (ab243906)

Formalin-fixed, paraffin-embedded HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling gamma H2A.X (phospho S139) using ab243906 at 1/100 dilution in ICC analysis. An HRP-conjugated goat-anti rabbit IgG was used as the secondary. DAB staining.

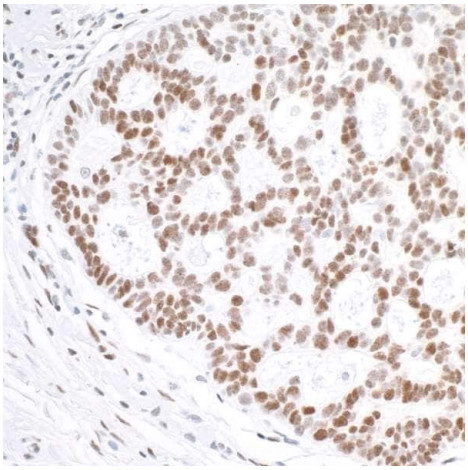
Left panel: Untreated HeLa cells

Right panel: Etoposide treated HeLa cells



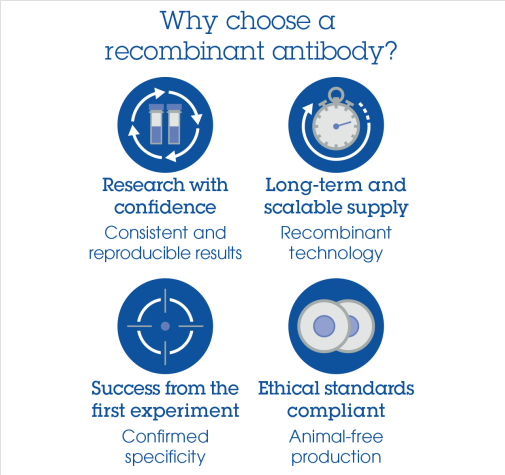
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [BLR053F] (ab243906)

Formalin-fixed, paraffin-embedded mouse CT26 colon carcinoma tissue stained for H2A.X (phospho S139) using ab243906 at 1/250 dilution in immunohistochemical analysis. A HRP-conjugated goat anti-rabbit antibody was used as the secondary. DAB staining.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-gamma H2A.X (phospho S139) antibody [BLR053F] (ab243906)

Formalin-fixed, paraffin-embedded human G203basal cell carcinoma tissue stained for H2A.X (phospho S139) using ab243906 at 1/250 dilution in immunohistochemical analysis. A HRP-conjugated goat anti-rabbit antibody was used as the secondary. DAB staining.



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