abcam

Product datasheet

Human H2A.X (phospho S139) In-Cell ELISA Kit (IR) ab131382

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Overview

Product name Human H2A.X (phospho S139) In-Cell ELISA Kit (IR)

Detection method IR

Precision

Intra-assay

Sample	n	Mean	SD	CV%
HeLa Cells				< 10%

Sample type Adherent cells, Suspension cells

Assay type Cell-based (qualitative)

Assay duration Multiple steps standard assay

Species reactivity **Reacts with:** Human

Product overview The H2A.X (phospho S139) Human In-Cell ELISA Kit (IR) (ab131382) is designed to study the induction of DNA damage in response to various stimuli. A rabbit monoclonal antibody specific to H2A.X phospho S139 is used in this high-throughput duplexing plate-based assay. H2A.X (phospho S139) is a reliable readout for double-stranded DNA breaks.

Plates are available in our ICE (In-Cell ELISA) Support Pack (**ab111542**) which can be bought seperately.

In-Cell ELISA (ICE) technology is used to perform quantitative immunocytochemistry of cultured cells with a near-infrared fluorescent dye-labeled detector antibody. The technique generates quantitative data with specificity similar to western blotting, but with much greater quantitative precision and higher throughput due to the greater dynamic range and linearity of direct fluorescence detection and the ability to run up to 96 samples in parallel.

This method rapidly fixes the cells in situ, stabilizing the in vivo levels of proteins and their posttranslational modifications, and thus essentially eliminates changes during sample handling, such as preparation of protein extracts. Finally, the H2A.X (phospho S139) signal can be normalized to cell amount, measured by the provided Janus Green whole-cell stain, to further increase the assay precision.

This product is designed for LI-COR® Odyssey® or Aerius® infrared imaging systems.

Notes

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform Microplate

Properties

Storage instructions Store at +4°C. Please refer to protocols.

Components	1 x 96 tests
1000X IRDye®-Labeled Secondary Antibody	1 x 24µl
100X Anti-H2A.X (pSer139) Rabbit Primary Antibody	1 x 120µl
100X Triton X-100	1 x 0.5ml
10X Blocking Solution	1 x 10ml
10X Phosphate Buffered Saline	1 x 100ml
400X Tween-20	1 x 2ml
1X Janus Green Stain	1 x 17ml

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.

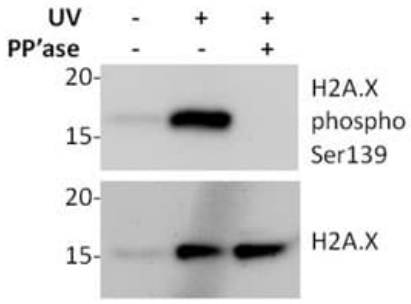
Domain The [ST]-Q motif constitutes a recognition sequence for kinases from the PI3/PI4-kinase family.

Post-translational modifications Phosphorylated on Ser-140 (to form gamma-H2AX 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 (By similarity). 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. Ubiquitination at Lys-14 and Lys-16 (H2AK13Ub and H2AK15Ub, respectively) in response to DNA damage is initiated by RNF168 that mediates monoubiquitination at these 2 sites, and 'Lys-63'-linked ubiquitin are then conjugated to monoubiquitin; RNF8 is able to extend 'Lys-63'-linked ubiquitin chains in vitro. H2AK119Ub and ionizing radiation-induced 'Lys-63'-linked ubiquitination (H2AK13Ub and H2AK15Ub) are distinct events.
Acetylation at Lys-37 increases in S and G2 phases. This modification has been proposed to play a role in DNA double-strand break repair.

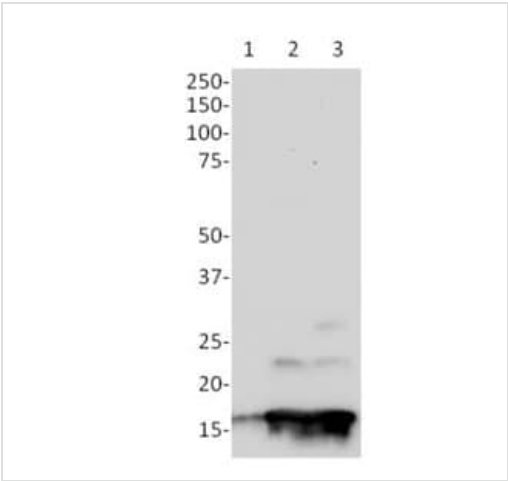
Cellular localization Nucleus. Chromosome.

Images



Western blot - Human H2A.X (phospho S139) In-Cell ELISA Kit (IR) (ab131382)

Antibody specificity demonstrated by Western Blot Analysis: H2A.X (phospho S139) is phospho-specific. Jurkat cells were stimulated with UV light exposure to induce H2A.X (phospho S139) and then the UV treated lysate was treated with lambda protein phosphatase. Top panel: H2A.X (phospho S139) is induced by UV treatment and the western blot band is sensitive to phosphatase treatment. Lower panel: In contrast, total H2A.X (**ab124781**) levels are not sensitive to phosphatase treatment.

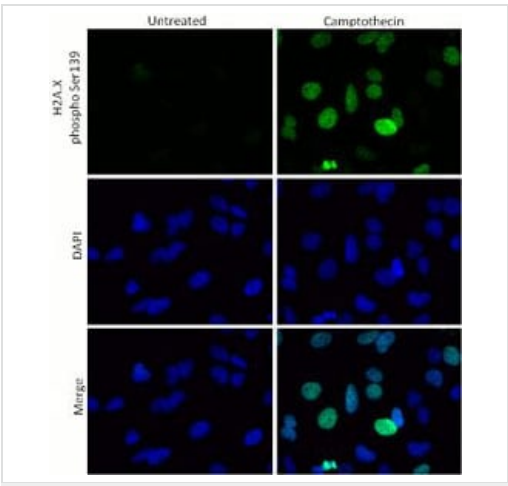


Western blot - Human H2A.X (phospho S139) In-Cell ELISA Kit (IR) (ab131382)

All lanes : Human H2A.X (phospho S139) In-Cell ELISA Kit (IR) (ab131382)

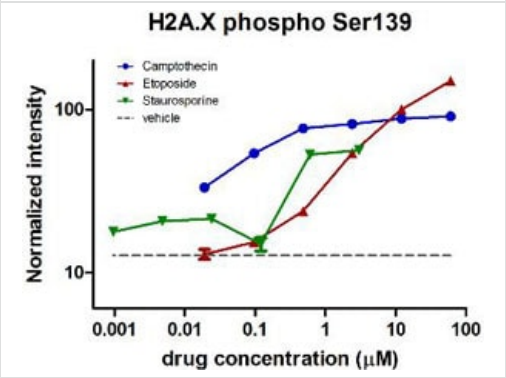
Lane 1 : Untreated cells
Lane 2 : Camptothecin treated cells
Lane 3 : UV exposed cells

Antibody specificity demonstrated by Western Blot Analysis. Whole cell lysates from Jurkat cells were analyzed by western blot with the H2A.X (phospho S139) antibody used in this assay kit.



Immunocytochemistry/ Immunofluorescence - Human H2A.X (phospho S139) In-Cell ELISA Kit (IR) (ab131382)

Specificity of H2A.X (phospho S139) antibodies demonstrated by immunocytochemistry. The primary antibody used in this assay kit was validated by staining HeLa cells treated with 10 µM Camptothecin or vehicle for 4 hours and imaged by fluorescent microscopy. Note the absence of H2A.X (phospho S139) in the untreated cells.



In-Cell ELISA - Human H2A.X (phospho S139) In-Cell ELISA Kit (IR) (ab131382)

Sample experiment using ab131382 on HeLa cells following drug treatment: H2A.X (phospho S139) readout. HeLa cells were treated for 4 hours with dose titrations of Camptothecin, Etoposide and Staurosporin. The dashed grey line indicates the vehicle control signal.

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