Datasheet for ABIN967669

anti-H2AFX antibody (pSer139)

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Overview

Quantity:	0.1 mg
Target:	H2AFX
Binding Specificity:	pSer139
Reactivity:	Human, Mouse
Host:	Mouse
Clonality:	Monoclonal
Conjugate:	This H2AFX antibody is un-conjugated
Application:	Western Blotting (WB), BioImaging (BI)

Product Details

Brand:	BD Pharmingen™
Immunogen:	Phosphorylated Human H2AX Peptide
Clone:	N1-431
Isotype:	IgG1 kappa
Cross-Reactivity:	Mouse (Murine)
Characteristics:	1. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing. 2. Triton is a trademark of the Dow Chemical Company. 3. Alexa Fluor® is a registered trademark of Molecular Probes, Inc., Eugene, OR. 4. Please refer to us for technical protocols.

Product Details

Purification:	The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Target Details

Target:	H2AFX
Alternative Name:	H2AX (H2AFX Products)
Background:	Histones are highly basic proteins that complex with DNA to form chromatin. The H2AX histone (~15 kDa calculated molecular weight) is a member of the H2A histone family whose members are components of nucleosomal histone octamers. Double-stranded breaks in DNA caused by replication errors, apoptosis, or other physiological processes (including, immunoglobulin and TCR gene recombinations) and DNA damage caused by ionizing radiation, UV light, or cytotoxic agents lead to phosphorylation of H2AX on serine 139. H2AX (pS139) is also referred to as H2AX (pS140) when the N-terminal methionine that is normally excised during posttranslational processing is included in amino acid sequence numbering. Kinases such as ataxia telangiectasia mutated (ATM) or ATM-Rad3-related (ATR) phosphorylate H2AX to induce its function. Phosphorylated H2AX (also termed, gamma-H2AX) functions to recruit and localize DNA repair proteins or cell cycle checkpoint factors to the DNA-damaged sites. In this way, phosphorylated H2AX promotes DNA repair and maintains genomic stability and thus helps prevent oncogenic transformations. Immunofluorescent staining and bioimaging analysis of cultured cells can be used to readily identify H2AX (pS139)-containing foci. As such, H2AX (pS139) immunofluorescence localization serves as a biomarker for nuclear sites of DNA damage (e.g., double-stranded DNA breaks) in affected cells. Synonyms: H2A.X, H2A/X, H2AFX, HIST5-2AX, gamma-H2AX, gamma-H2AX, H2AX (pS140)
Pathways:	Telomere Maintenance, DNA Damage Repair, Positive Regulation of Response to DNA Damage Stimulus

Application Details

Application Notes:	Recommended Assay Procedure for Bioimaging: 1. Seed the cells in appropriate culture medium at an appropriate cell density in an 96-well Imaging Plate , and culture overnight to 48 hours. 2. Remove the culture medium from the wells, and wash (one to two times) with 100 myl of 1× PBS. 3. Fix the cells by adding 100 µl of fresh 3.7% Formaldehyde in PBS or fixation buffer to each well and incubating for 10 minutes at room temperature (RT).

Application Details

	4. Remove the fixative from the wells, and wash the wells (one to two times) with 100 myl of 1× PBS. 5. Permeabilize the cells using either cold methanol (a), Triton™ X-100 (b), or Saponin (c): a. Add 100 µl of -20°C 90% methanol to each well and incubate for 5 minutes at RT. b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT. c. Add 100 µl of 1× Perm/Wash buffer to each well and incubate for 15 to 30 minutes at RT. Continue to use 1× Perm/Wash buffer for all subsequent wash and dilutions steps. 6. Remove the permeabilization buffer from the wells, and wash one to two times with 100 myl of appropriate buffer (either 1× PBS or 1× Perm/Wash buffer, see step 5.c.). 7. Optional blocking step: Remove the wash buffers, and block the cells by adding 100 µl of blocking buffer or 3% FBS in appropriate dilution buffer to each well and incubating for 15 to 30 minutes at RT. 8. Dilute the antibody to its optimal working concentration in appropriate dilution buffer. Titrate purified (unconjugated) antibodies and second-step reagents to determine the optimal concentration. If using a Bioimaging Certified antibody conjugate, dilute it 1:10. 9. Add 50 µl of diluted antibody per well and incubate for 60 minutes at RT. Incubate in the dark if using fluorescently labeled antibodies. 10. Remove the antibody, and wash the wells three times with 100 myl of wash buffer. An optional detergent wash (100 myl of 0.05% Tween in 1× PBS) can be included prior to the regular wash steps. 11. If the antibody being used is fluorescently labeled, then move to step 12. Otherwise, if using a purified unlabeled antibody, repeat steps 8 to 10 with a fluorescently labeled second-step reagent to detect the purified antibody. 12. After the final wash, counter-stain the nuclei by adding 100 ml of a 2 mg/ml solution of Hoechst 33342 in 1× PBS to each well at least 15 minutes before imaging. 13. View and analyze the cells on an appropriate imaging instrument.
Comment:	Related Products: ABIN967389
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	0.5 mg/mL
Buffer:	Aqueous buffered solution containing ≤0.09 % sodium azide.

Handling

Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.
Storage:	4 °C
Storage Comment:	Store undiluted at 4°C.

Publications

Product cited in:

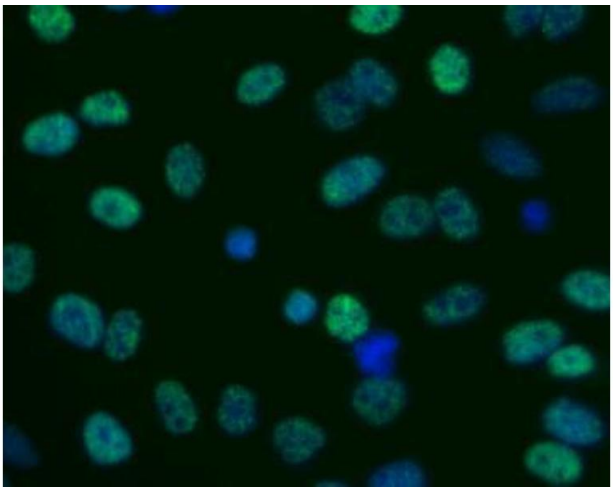
Kuo, Yang: "Gamma-H2AX - a novel biomarker for DNA double-strand breaks." in: **In vivo (Athens, Greece)**, Vol. 22, Issue 3, pp. 305-9, (2008) (PubMed).

Fernandez-Capetillo, Lee, Nussenzweig, Nussenzweig: "H2AX: the histone guardian of the genome." in: **DNA repair**, Vol. 3, Issue 8-9, pp. 959-67, (2004) (PubMed).

Burma, Chen, Murphy, Kurimasa, Chen: "ATM phosphorylates histone H2AX in response to DNA double-strand breaks." in: **The Journal of biological chemistry**, Vol. 276, Issue 45, pp. 42462-7, (2001) (PubMed).

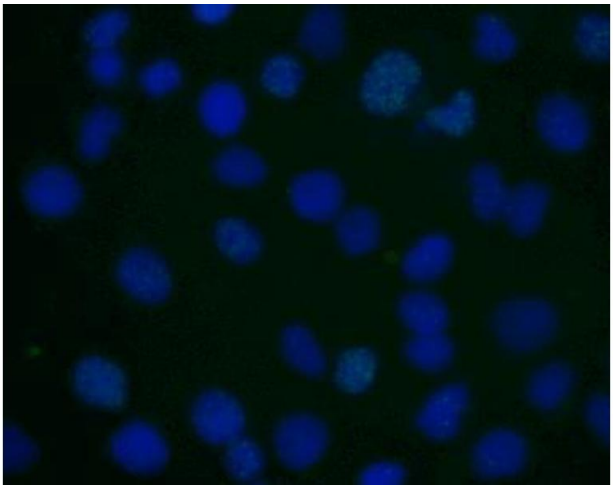
Rogakou, Nieves-Neira, Boon, Pommier, Bonner: "Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139." in: **The Journal of biological chemistry**, Vol. 275, Issue 13, pp. 9390-5, (2000) (PubMed).

Rogakou, Pilch, Orr, Ivanova, Bonner: "DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139." in: **The Journal of biological chemistry**, Vol. 273, Issue 10, pp. 5858-68, (1998) (PubMed).



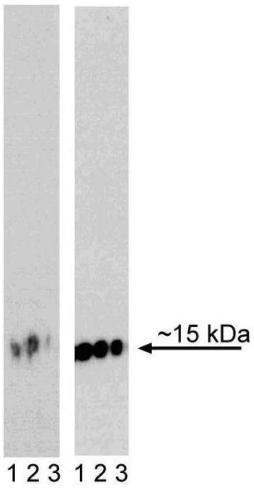
Immunofluorescence

Image 1. Immunofluorescent staining of human cell line



Immunofluorescence

Image 2. Immunofluorescent staining of human cell line. HeLa cells (ATCC CCL-2) were seeded in a 96-well Imaging Plate at ~10,000 cells per well. After overnight culture, the cells were exposed to 2400 Joules UV irradiation (right image) or untreated (left image) and then allowed to recover for 30-60 minutes at 37°C. The cells were fixed, permeabilized with cold methanol, and stained with Purified Mouse anti-H2AX (pS139) followed by Alexa Fluor® 488 goat anti-mouse IgG (Invitrogen, pseudo colored green) according to the Recommended Assay Procedure. Cell nuclei were counterstained with Hoechst 33342 (pseudo colored blue). The images were captured on a BD Pathway™ 435 high-content Bioimager system using a 20X objective and merged using BD AttoVision™ software. This antibody also worked with the Saponin and the Triton™ X-100 Perm/Wash protocols (see Recommended Assay Procedure, Bioimaging protocol link).



Western Blotting

Image 3. Western blot analysis of H2AX (pS139) in transformed human epithelioid carcinoma. Lysates from control (first panel) and Staurosporine-treated (second panel) HeLa cells (ATCC CCL-2) were probed with Purified Mouse anti-H2AX (pS139) monoclonal antibody at concentrations of 0.25, 0.125, and 0.06 µg/ml (Lanes 1, 2, and 3, respectively). H2AX (pS139)is identified as a band of ~15 kDa with increased density in the treated cells.

Please check the product details page for more images. Overall 4 images are available for ABIN967669.