

**#9718** Store at -20°C

# Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb

100 µl  
 (10 western blots)



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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

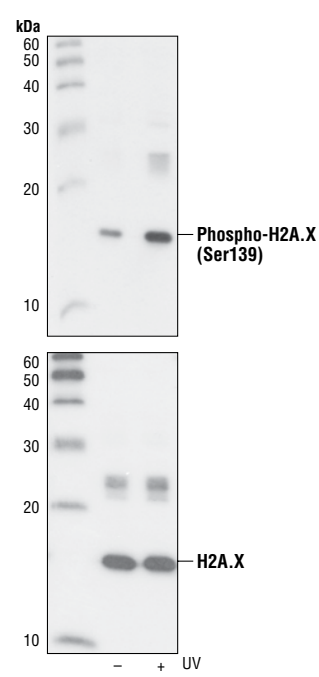
**Entrez-Gene ID #** 3014  
**Swiss-Prot Acc. #** P16104

Applications	Species Cross-Reactivity*	Molecular Wt.	Isotype
W, IHC-P, IF-IC, F Endogenous	H, M, R, Mk	15 kDa	Rabbit IgG**

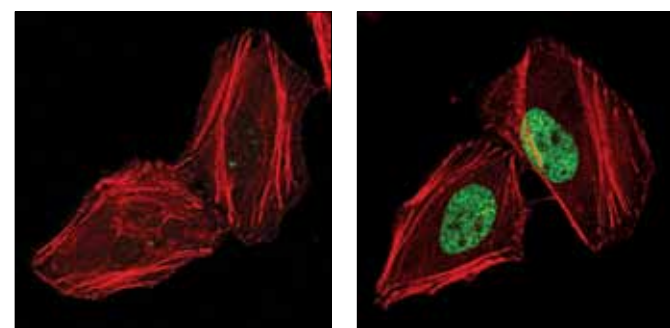
**Background:** Histone H2A.X is a variant histone that represents approximately 10% of the total H2A histone proteins in normal human fibroblasts (1). H2A.X is required for checkpoint-mediated cell cycle arrest and DNA repair following double-stranded DNA breaks (1). DNA damage, caused by ionizing radiation, UV-light, or radiomimetic agents, results in rapid phosphorylation of H2A.X at Ser139 by PI3K-like kinases, including ATM, ATR and DNA-PK (2,3). Within minutes following DNA damage, H2A.X is phosphorylated on Ser139 at sites of DNA damage (4). This very early event in the DNA-damage response is required for recruitment of a multitude of DNA-damage response proteins, including MDC1, NBS1, RAD50, MRE11, 53BP1 and BRCA1 (1). In addition to its role in DNA-damage repair, H2A.X is required for DNA fragmentation during apoptosis and is phosphorylated by various kinases in response to apoptotic signals. H2A.X is phosphorylated on Ser139 by DNA-PK in response to cell death receptor activation, c-Jun N-terminal Kinase (JNK1) in response to UV-A irradiation, and p38 MAPK in response to serum starvation (5-8). H2A.X is constitutively phosphorylated on Tyr142 in undamaged cells by WSTF (Williams-Beuren syndrome transcription factor) (9,10). Upon DNA damage, and concurrent with phosphorylation of Ser139, Tyr142 is dephosphorylated at sites of DNA damage by recruited EYA1 and EYA3 phosphatases (9). While phosphorylation of Ser139 facilitates the recruitment of DNA repair proteins and apoptotic proteins to sites of DNA damage, phosphorylation of Tyr142 appears to determine which set of proteins are recruited. Phosphorylation of H2A.X on Tyr142 inhibits the recruitment of DNA repair proteins and promotes binding of pro-apoptotic factors such as JNK1 (9). Mouse embryonic fibroblasts expressing only mutant H2A.X Y142F, which favors recruitment of DNA repair proteins over apoptotic proteins, show a reduced apoptotic response to ionizing radiation (9). Thus, it appears that the balance of H2A.X Tyr142 phosphorylation and dephosphorylation provides a switch mechanism to determine cell fate after DNA damage.

**Specificity/Sensitivity:** Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb detects endogenous levels of H2A.X only when phosphorylated at serine 139.

**Source/Purification:** Monoclonal antibody is produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser139 of human H2A.X.



Western blot analysis of extracts from untreated or UV-treated 293 cells, using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (upper) or Histone H2A.X Antibody #2595 (lower).



Confocal immunofluorescent analysis of HeLa cells, untreated (left) or UV-treated (right), using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (green). Actin filaments have been labeled with DY-554 phalloidin (red).

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

**\*Species cross-reactivity is determined by western blot.**  
**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

**Recommended Antibody Dilutions:**

Western blotting	1:1000
Immunohistochemistry (Paraffin)	1:480†
Unmasking buffer:	Citrate
Antibody diluent:	SignalStain® Antibody Diluent #8112
Detection reagent:	SignalStain® Boost (HRP, Rabbit) #8114
†Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.	
Immunofluorescence (IF-IC)	1:400
Flow Cytometry	1:400

**For application specific protocols please see the web page for this product at [www.cellsignaling.com](http://www.cellsignaling.com).**  
**Please visit [www.cellsignaling.com](http://www.cellsignaling.com) for a complete listing of recommended companion products.**

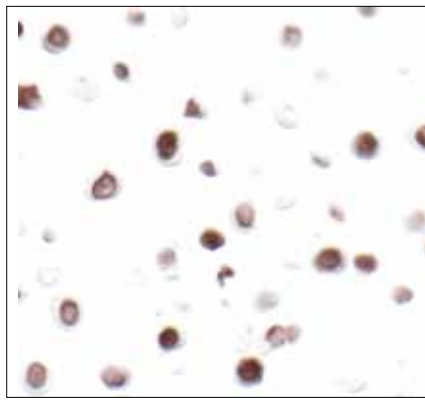
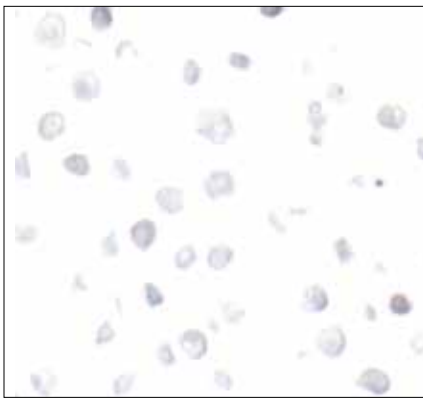
**Background References:**

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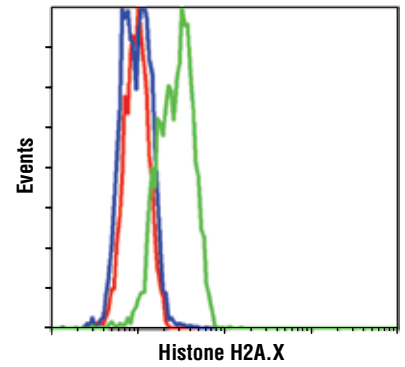
**IMPORTANT: For western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide  
**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine  
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

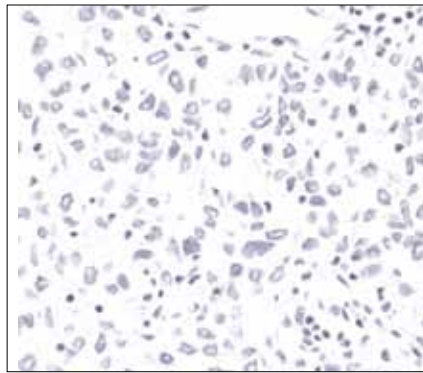
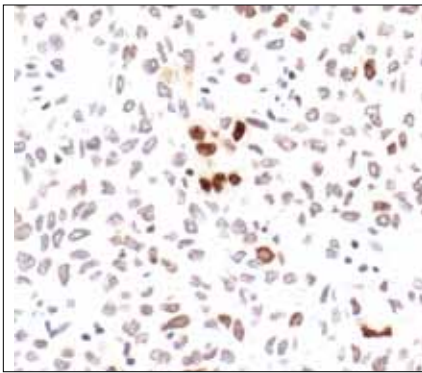
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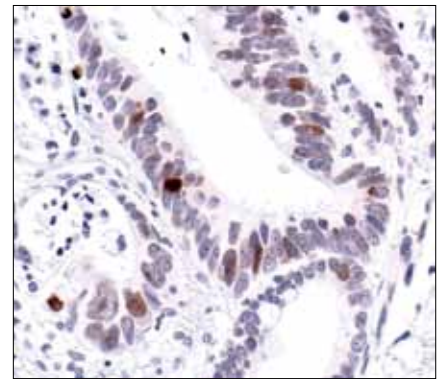
Immunohistochemical analysis of paraffin-embedded HT-29 cells untreated (left) or UV-treated (right), using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb.



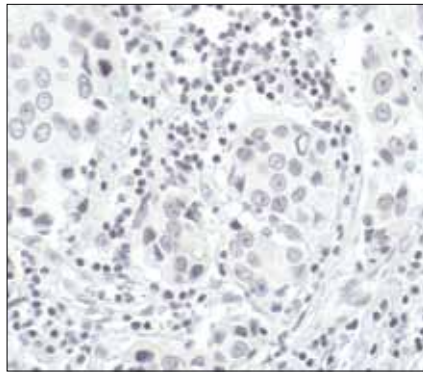
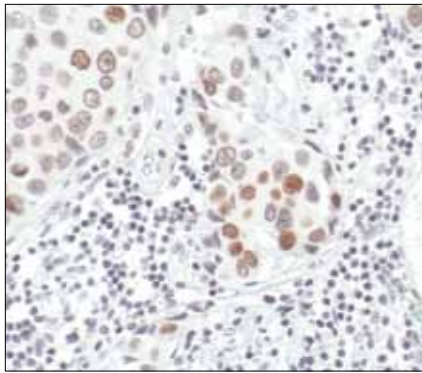
Flow cytometric analysis of HeLa cells untreated (blue) or UV-treated (green), using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb compared to a nonspecific negative control antibody (red).



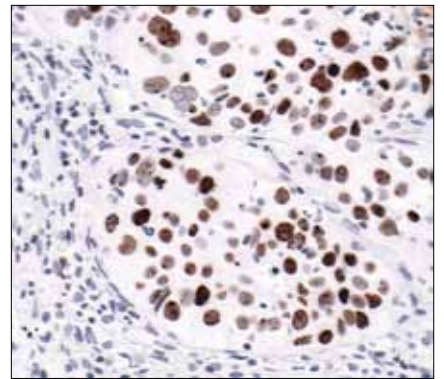
Immunohistochemical analysis of paraffin-embedded human lung carcinoma untreated (left) or lambda-phosphatase-treated (right), using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human colon carcinoma, using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb.



Immunohistochemical analysis of paraffin-embedded human breast carcinoma, using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb in the presence of control peptide (left) or Phospho-Histone H2A.X (Ser139) Blocking Peptide #1260 (right).



Immunohistochemical analysis of paraffin-embedded human lung carcinoma, using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb, showing nuclear localization.