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

**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 EY1 and EY3 phosphatases (9). While phosphorylation of Ser139 facilitates 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.

**Recommended Antibody Dilutions:**

Western blotting: 1:1000

Immunohistochemistry (Paraffin): 1:480

Unmasking buffer: Citrate

Antibody diluent: SignalStain® Boost (HRP, Rabbit) #6114

Optimal IHC dilutions determined using SignalStain® Boost IHC Detection Reagent.

Immunofluorescence (1:IC): 1:400

Flow Cytometry: 1:200

For application specific protocols please see the web page for this product at www.cellsignal.com.

**Please visit www.cellsignal.com for a complete listing of recommended companion products.**
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 treated with UV (100 mJ, 2 hr recovery; green) using Phospho-H2A.X (Ser139) (20E3) Rabbit mAb (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® isotype control #3900 (dashed lines). Anti-rabbit IgG (H+L), F(ab')2 Fragment (Alexa Fluor® 488 Conjugate) #4412 was used as a secondary antibody.

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

Background References: