**Phospho-Histone H2A.X (Ser139) Antibody**

**Applications**
- Western, IF-IC, F

**Species Cross-Reactivity**
- H, M, R, Mk

**Molecular Wt.**
- 15 kDa

**Source**
- Rabbit

---

**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 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 H2A.X on Tyr142 inhibits 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-H2A.X (Ser139) Antibody detects endogenous levels of H2A.X only when phosphorylated at Ser139.

**Recommended Antibody Dilutions:**
- Western blotting 1:1000
- Immunofluorescence (IF-IC) 1:800
- Flow Cytometry 1:800

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.

**Background References:**

**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.
Confocal microscopic images of HeLa cells, UV treated (A) and untreated (B), showing nuclear stain with Phospho-Histone H2A.X (Ser139) Antibody (red) and Phospho-SAPK/JNK (Thr183/Tyr185) (G9) Mouse mAb #9255 (green).

Flow cytometric analysis of HeLa cells, untreated (blue) and UV-treated (green), using Phospho-Histone H2A.X (Ser139) Antibody compared with a nonspecific negative control antibody (red).