Revision 1

Store at -20C

#5438

# Phospho-Histone H2A.X (Ser139/Tyr142) Antibody

Cell Signaling TECHNOLOGY®

**Orders:** 877-616-CELL (2355) orders@cellsignal.com

**Support:** 877-678-TECH (8324)

**Web:** info@cellsignal.com cellsignal.com

3 Trask Lane | Danvers | Massachusetts | 01923 | USA

**For Research Use Only. Not for Use in Diagnostic Procedures.**

| Applications: | Reactivity: | Sensitivity: | MW (kDa): | Source/Isotype: | UniProt ID: | Entrez-Gene Id: |
|---|---|---|---|---|---|---|
| W, IP, IF-IC, FC-FP | H M R Mk | Endogenous | 15 | Rabbit | #P16104 | 3014 |

## Product Usage Information

| Application | Dilution |
|---|---|
| Western Blotting | 1:1000 |
| Immunoprecipitation | 1:50 |
| Immunofluorescence (Immunocytochemistry) | 1:100 |
| Flow Cytometry (Fixed/Permeabilized) | 1:200 |

## 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.

## Specificity/Sensitivity

Phospho-Histone H2A.X (Ser139/Tyr142) Antibody recognizes endogenous levels of histone H2A.X protein only when phosphorylated at Ser139 or Tyr142, or dually phosphorylated on both residues. This antibody does not cross-react with other histone proteins.

## Source / Purification

Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser139 and Tyr142 of human H2A.X protein. Antibodies are purified by protein A and peptide affinity chromatography.

## 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 at Ser139 at sites of DNA damage to generate γ-H2A.X (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 at 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 at Ser139 facilitates the recruitment of DNA repair proteins and apoptotic proteins to sites of DNA damage, phosphorylation at Tyr142 appears to determine which set of proteins are recruited. Phosphorylation of H2A.X at 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.

## Background References

1. Yuan, J. et al. (2010) *FEBS Lett* 584, 3717-24.
2. Rogakou, E.P. et al. (1998) *J Biol Chem* 273, 5858-68.
3. Burma, S. et al. (2001) *J Biol Chem* 276, 42462-7.
4. Rogakou, E.P. et al. (1999) *J Cell Biol* 146, 905-16.
5. Mukherjee, B. et al. (2006) *DNA Repair (Amst)* 5, 575-90.
6. Solier, S. et al. (2009) *Mol Cell Biol* 29, 68-82.
7. Lu, C. et al. (2006) *Mol Cell* 23, 121-32.
8. Lu, C. et al. (2008) *FEBS Lett* 582, 2703-8.
9. Cook, P.J. et al. (2009) *Nature* 458, 591-6.
10. Xiao, A. et al. (2009) *Nature* 457, 57-62.

## Species Reactivity

Species reactivity is determined by testing in at least one approved application (e.g., western blot).

## Western Blot Buffer

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

## Applications Key

**W:** Western Blotting **IP:** Immunoprecipitation **IF-IC:** Immunofluorescence (Immunocytochemistry) **FC-FP:** Flow Cytometry (Fixed/Permeabilized)

## Cross-Reactivity Key

**H:** Human **M:** Mouse **R:** Rat **Mk:** Monkey

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