## **Histone H2A.X Antibody**



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

Applications: Reactivity: Sensitivity: MW (kDa): Source: **UniProt ID:** Entrez-Gene Id: WB HMRMk Endogenous 15 Rabbit #P16104 3014 **Product Usage** Application Dilution

Information Western Blotting 1:1000

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**Histone H2A.X Antibody detects endogenous levels of histone H2A.X protein independent of phosphorylation and ubiquitination.

**Source / Purification**Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to residues near the carboxy-terminus of human histone H2A.X. Antibodies are purified by protein A and peptide affinity chromatography.

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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 (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**

**Background** 

- 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.

1/1/24. 11:40 AM

**Applications Key** 

**Cross-Reactivity Key** 

Histone H2A.X Antibody (#2595) Datasheet Without Images Cell Signaling Technology

WB: Western Blotting

H: human M: mouse R: rat Hm: hamster Mk: monkey Vir: virus 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

GP: Guinea Pig Rab: rabbit All: all species expected

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