Store at -20°C

PhosphoPlus® Histone H2A.X (Ser139) Antibody Duet

#45456

Cell Signaling TECHNOLOGY®

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New 04/21

Entrez-Gene ID #3014
UniProt ID #P16104

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb	9718	100 µl	15 kDa	Rabbit IgG
Histone H2A.X (D17A3) XP® Rabbit mAb	7631	100 µl	15 kDa	Rabbit IgG

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

Description: PhosphoPlus® Duets from Cell Signaling Technology (CST) provide a means to assess protein activation status. Each Duet contains an activation-state and total protein antibody to your target of interest. These antibodies have been selected from CST's product offering based upon superior performance in specified applications.

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 (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.

Specificity/Sensitivity: Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb detects endogenous levels of H2A.X only when phosphorylated at Ser139. Histone H2A.X (D17A3) XP® Rabbit mAb recognizes endogenous levels of total histone H2A.X protein. This antibody does not cross-react with other histone H2A proteins.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser139 of human histone H2A.X or with a synthetic peptide corresponding to residues surrounding Val124 of human histone H2A.X protein.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

For product specific protocols and a complete listing of recommended companion products please see the product web page at www.cellsignal.com.

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.

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Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide **Species Cross-Reactivity:** **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.