Store at 4°C

#9719

Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 488 Conjugate)

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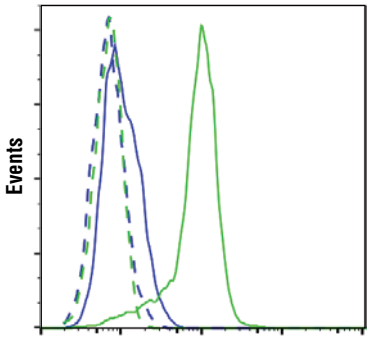
Entrez-Gene ID #3014
UniProt ID #P16104

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

Applications	Species Cross-Reactivity*	Isotype
F Endogenous	H, M	Rabbit IgG

Description: This Cell Signaling Technology antibody is conjugated to Alexa Fluor® 488 fluorescent dye and tested in-house for direct flow cytometry and immunofluorescent analysis in human cells. The antibody is expected to exhibit the same species cross-reactivity as the unconjugated Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb #9718.

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 phosphor-ylation 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 phosphor-ylation and dephosphorylation provides a switch mechanism to determine cell fate after DNA damage.



Phospho-Histone H2A.X (Ser139)
(Alexa Fluor® 488 Conjugate)

Flow cytometric analysis of HeLa cells, untreated (blue) or treated with with UV (100 mJ/cm2 with 3 hr recovery; green) using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb (Alexa Fluor® 488 Conjugate) (solid lines) or concentration-matched Rabbit (DA1E) mAb IgG XP® Isotype Control (Alexa Fluor® 488 Conjugate) #2975 (dashed lines).

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. The antibody was conjugated to Alexa Fluor® 488 under optimal conditions with an F/P ratio of 2-5.

Rabbit Monoclonals Produced Using Epitomics® Technology, U.S. Patent No. 5,675,063.Alexa Fluor® is a registered trademark of Molecular Probes, Inc.

Storage: Supplied in PBS (pH 7.2), less than 0.1% sodium azide and 2 mg/ml BSA. Store at 4°C. Do not aliquot the antibody. Protect from light. Do not freeze.

***Species cross-reactivity is determined by western blot.**

HRP-conjugated antibodies do not require incubation with a secondary antibody.

Recommended Antibody Dilutions:

Flow Cytometry	1:50

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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