APC/Fire™ 750 anti-H2A.X Phospho (Ser139) Antibody

Catalog# / Size	613421 / 25 tests 613422 / 100 tests
Clone	2F3
Regulatory Status	RUO
Other Names	H2A.x, H2a/x, Histone 2A, Histone 2A.X, Gamma-H2AX
Isotype	Mouse IgG1, κ
Description	H2A.X is a 14 kD basal histone and a member of the H2 histone family. This nuclear protein is synthesized in the G1 and S phase of the cell cycle and is known to be important for DNA repair and maintaining genomic stability and for recombination between immunoglobulin switch regions. H2A.X becomes phosphorylated on serine 139 after double-stranded DNA breaks. Phosphorylated H2A.X promotes DNA repair and maintains genomic stability. The 2F3 monoclonal antibody reacts with phosphorylated human H2A.X (Ser139) and has been shown to be useful for Western blotting, immunofluorescence and flow cytometry.

Product Details

Verified Reactivity	Human, Mouse
Antibody Type	Monoclonal
Host Species	Mouse
Immunogen	Modified peptide
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA)
Concentration	Lot-specific (to obtain lot-specific concentration, please enter the lot number in our Concentration and Expiration Lookup or Certificate of Analysis online tools.)
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C, and protected from prolonged exposure to light. **Do not freeze.**
Application	ICFC - *Quality tested*
Recommended Usage	Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood. * APC/Fire™ 750 has a maximum excitation of 650 nm and a maximum emission of 787 nm.
Excitation Laser	Red Laser (633 nm)
Application Notes	**Additional reported applications (for the relevant formats of this clone) include:** immunohistochemistry on paraffin embedded sections[2], immunofluorescence microscopy[3-9], Western blotting [10-12], and flow cytometry[1,13]. Clone 2F3 cross-reacts with mouse[4]. **Intracellular staining protocol for Anti-H2A.X-Phosphorylated (Ser139) Antibody for Flow Cytometry** 1. Prepare 70% absolute ethanol. Chill solution by storing at -20°C. 2. Prepare cells of interest. 3. Wash 1X with PBS, centrifuge at 350g for 5 min. 4. Discard the supernatant and vortex to loosen cell pellet. 5. Add pre-cooled 70% ethanol drop by drop, while vortexing. 6. Incubate at -20°C for 60 minutes. 7. Wash 3X with BioLegend Cell Staining Buffer and resuspend the cells at 0.5-1 X 10^7 cells/ml in the cell staining buffer. 8. Perform immunofluorescent staining for flow cytometry.
Application References	1. Jha JC, *et al.* 2013. *J. Virol.* 87:5255. (FC) PubMed

(PubMed link indicates BioLegend citation)

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4. Xiao R, *et al.* 2007. *Mol Cell Biol.*27:5393. (IF) PubMed
5. Rossi DJ, *et al.* 2007. *Nature*. 447:725. (IF) PubMed
6. Loidl J, *et al.* 2009. *Mol Cell Biol.* 20:2048. (IF) PubMed
7. Beels L, *et al.* 2009. *Circulation.* 120:1903. (IF) PubMed
8. Suzuki K, *et al.* 2010. *Nucleic Acids Res.* 38:e129. (IF) PubMed
9. Lukaszewicz A. 2010. *Chromasoma* Apr 27. [Epub ahead of print] (IF) PubMed
10. Yamada C, *et al.* 2010 *J. Biol. Chem.* 285:16693. (WB) PubMed
11. Bu Y, *et al.* 2010, *Biochem Biophys Res Commun.* 397:157. (WB) PubMed
12. Massignan T, *et al.* 2010. *J. Biol Chem.* 285:7752. (WB) PubMed
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16. Yoshihara Y, *et al.* 2012. *Biochem Biophys Res Cmmun.* 421:57. PubMed
17. Titus S, *et al.* 2013. *Sci Transl Med.* 13:21. PubMed
18. Crown KN, *et al.* 2013. *G3.* 6:1927. PubMed
19. Schenkwein D, *et al.* 2013. *Nucleic Acids Res.* 41:e61. PubMed
20. Zhadanova NS, *et al.* 2014. *Mol Cell Biol.* 34:2786. PubMed
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22. Maya-Mendoza A, *et al.* 2015. *Mol Oncol.* 9:601. PubMed

RRID

AB_2715786 (BioLegend Cat. No. 613421)
AB_2715787 (BioLegend Cat. No. 613422)

Antigen Details

Structure

Basal histone, H2 histone family; 14 kD

Distribution

Nuclear

Function

Phosphorylated H2AX promotes DNA repair and maintains genomic stability. Important for recombination between immunoglobulin switch regions

Modification

Phosphorylation on Ser139 after double-stranded DNA breaks

Biology Area

Cell Biology, Chromatin Remodeling/Epigenetics, DNA Repair/Replication, Neuroscience

Molecular Family

Phospho-Proteins

Antigen References

1. Mannironi C, *et al.*1989. *Nucleic Acids Res.* 17:9113.
2. Celeste A, *et al.* 2002. *Science* 296:922.
3. Bassing CH, *et al.* 2002. *Proc. Natl. Acad. Sci. USA* 99:8173.
4. Reina-San-Martin B, *et al.* 2003. *J. Exp. Med.* 197:1767.

Regulation

Synthesized in G1 and S-phase of cell cycle

Gene ID

3014

Related Protocols

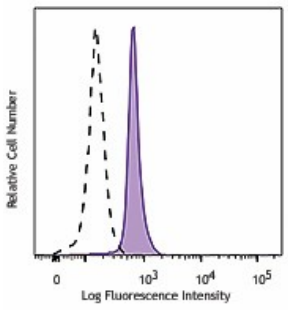
Surface and Intracellular Cytokine Staining for Flow Cytometry - Video

Intracellular Flow Cytometry Staining Protocol

Other Formats

Purified anti-H2A.X Phospho (Ser139), FITC anti-H2A.X Phospho (Ser139), Alexa Fluor® 488 anti-H2A.X Phospho (Ser139), Alexa Fluor® 647 anti-H2A.X Phospho (Ser139), Alexa Fluor® 594 anti-H2A.X Phospho (Ser139), PE anti-H2A.X Phospho (Ser139), PerCP/Cyanine5.5 anti-H2A.X-Phosphorylated (Ser139), Direct-Blot™ HRP anti-H2A.X-Phosphorylated (Ser139), APC anti-H2A.X-Phosphorylated (Ser139), PE/Cyanine7 anti-H2A.X Phospho (Ser139), APC/Fire™ 750 anti-H2A.X Phospho (Ser139)

Product Data



Nocodazole treated HeLa cells (24 hours) were fixed and permeabilized with cold 70% ethanol, then intracellularly stained with anti-H2A.X Phospho (Ser139) (clone 2F3) APC/Fire™ 750 (filled histogram) or mouse IgG1, κ APC/Fire™ 750 (open histogram).

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