Alexa Fluor® 594 anti-H2A.X Phospho (Ser139) Antibody

Catalog# / Size	613410 / 100 µg
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.
Preparation	The antibody was purified by affinity chromatography and conjugated with Alexa Fluor® 594 under optimal conditions.
Concentration	0.5 mg/ml
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	ICC - *Quality tested*
Recommended Usage	Each lot of this antibody is quality control tested by immunocytochemistry. For immunocytochemistry, a concentration range of 1.25 - 5.0 µg/ml is recommended. It is recommended that the reagent be titrated for optimal performance for each application. * Alexa Fluor® 594 has an excitation maximum of 590 nm, and a maximum emission of 617 nm. Alexa Fluor® and Pacific Blue™ are trademarks of Life Technologies Corporation. View full statement regarding label licenses
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

(PubMed link indicates BioLegend citation)

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9. Lukaszewicz A. 2010. *Chromasoma* Apr 27. [Epub ahead of print] (IF) PubMed
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21. Horrell SA, *et al.* 2014. *Eukaryot Cell.* 13:1300. PubMed
22. Maya-Mendoza A, *et al.* 2015. *Mol Oncol.* 9:601. PubMed

Product Citations

1. Aury–Landas J, *et al.* 2019. Cell Physiol Biochem. 53:731. PubMed
2. Li J, *et al.* 2022. J Clin Invest. Online ahead of print. PubMed
3. Zhao B, *et al.* 2020. Nat Commun. 11:908. PubMed

RRID AB_2616774 (BioLegend Cat. No. 613410)

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

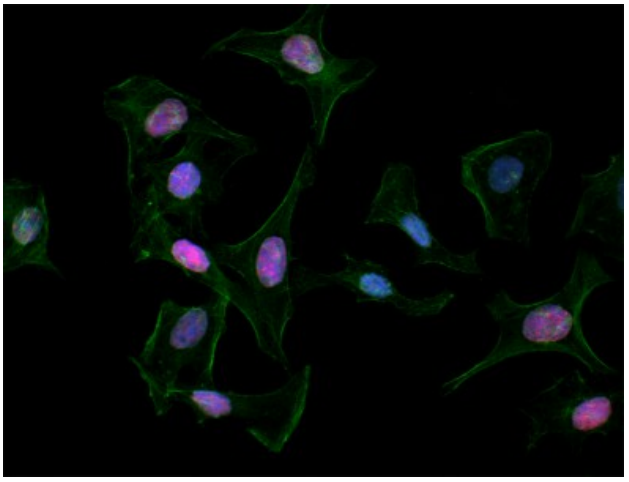
Related Protocols

Immunocytochemistry 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



HeLa cells were fixed with 1% paraformaldehyde (PFA) for ten minutes, permeabilized with 0.5% Triton X-100 for ten minutes, and blocked with 5% FBS for 30 minutes. Then the cells were intracellularly stained with 5 µg/ml of Alexa Fluor® 594 anti-H2A.X Phospho (Ser139) (clone 2F3) (red) in 5% FBS overnight followed by Alexa Fluor® 488 Phalloidin (green) staining for 20 minutes at 4°C. Nuclei were counterstained with DAPI (blue). The image was captured with 40X objective.

BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587