

Alexa Fluor® 647 anti-H2A.X Phospho (Ser139)

Catalog # / Size: 3667035 / 25 tests
3667040 / 100 tests

Clone: 2F3

Isotype: Mouse IgG1, κ

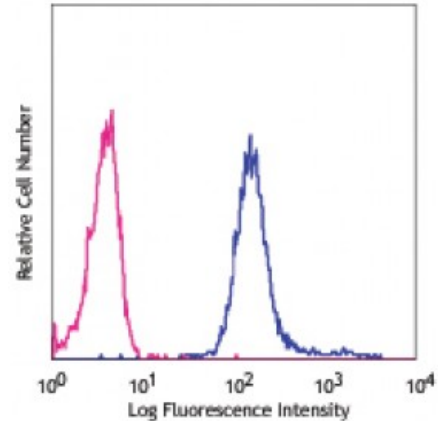
Immunogen: Modified peptide

Reactivity: Human, Mouse

Preparation: The antibody was purified by affinity chromatography, and conjugated with Alexa Fluor® 647 under optimal conditions.

Formulation: Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and 0.2% (w/v) BSA (origin USA).

Concentration: Lot-specific



Nocodazole-treated HeLa cells intracellularly stained with 2F3 Alexa Fluor® 647

Applications:

Applications: Immunofluorescence

Recommended Usage: Each lot of this antibody is quality control tested by immunofluorescent intracellular staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 microL per million cells. It is recommended that the reagent be titrated for optimal performance for each application.

* Alexa Fluor® 647 has a maximum emission of 668 nm when it is excited at 633nm / 635nm.

Application Notes: **Additional reported applications (for the relevant formats of this clone) include:** immunohistochemistry on paraffin embedded sections², immunofluorescence microscopy³⁻⁹, Western blotting¹⁰⁻¹², and flow cytometry^{1,13}. Clone 2F3 cross-reacts with mouse⁴.

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: resuspend with PBS, then pellet cells by centrifugation (250Xg, 5min)
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 10⁷ cells/ml in the cell staining buffer.
8. Perform immunofluorescent staining for flow cytometry.

Application References:

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 11. Bu Y, et al. 2010, *Biochem Biophys Res Commun.* 397:157. (WB) [PubMed](#)
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 13. Banath JP, et al. 2010. *BMC Cancer* 10:4 (FC)
 14. Zhang M., et al. 2011. *Cancer Res.* 23:7155. [PubMed](#)
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 17. Titus S, et al. 2013. *Sci Transl Med.* 13:21. [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](#)
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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.

- 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-Mar