BioLegend
The path to legendary discovery™

Product Data Sheet

Alexa Fluor® 488 anti-H2A.X-Phosphorylated (Ser139)

Catalog # / Size: 613405 / 25 tests
613406 / 100 tests

Clone: 2F3

Isotype: Mouse IgG1, κ

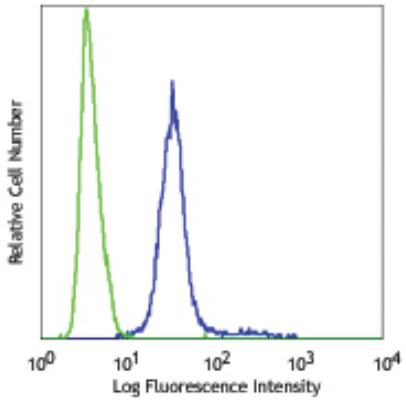
Immunogen: Modified peptide

Reactivity: Human

Preparation: The antibody was purified by affinity chromatography, and conjugated with Alexa Fluor® 488 under optimal conditions. The solution is free of unconjugated Alexa Fluor® 488.

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

Storage: The antibody solution should be stored undiluted at 4°C and protected from prolonged exposure to light. **Do not freeze.**



Nocodazole-treated Hela cells intracellularly stained with 2F3 Alexa Flour® 488

Applications:

Applications: ICFC - *Quality tested*
IF - *Quality tested*

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

* Alexa Fluor® 488 has a maximum emission of 519 nm when it is excited at 488 nm.
** Alexa Fluor® is a registered trademark of Molecular Probes, Inc. Alexa Fluor® dye antibody conjugates are sold under license from Molecular Probes, Inc. for research use only, except for use in combination with microarrays and high content screening, and are covered by pending and issued patents.

Application Notes: **Intracellular staining protocol for Anti-H2A.X-Phosphorylated (Ser139) Antibody for Flow Cytometry**

1. Prepare 70% absolute ethanol. Chill solution by storing at -20C.
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 -20C for 60 minutes.
7. Wash 3X with BioLegend Cell Staining Buffer and resuspend the cells at 0.5-1 X 10^7/ml in the cell staining buffer.
8. Perform immunofluorescent staining. **Additional reported applications (for the relevant formats of this clone) include:** immunohistochemistry on paraffin embedded sections[2], immunofluorescence microscopy[3-9], western blot [10-12], and flow cytometry[1,13].

Application References:
1. Moiseenko V, *et al.* 2008 *Radiat Oncol* 3:18 (FC)
2. Akbay A,*et al.* 2008. *Am J Pathol.* 173:536. (IHC) PubMed
3. Mochizuki K, *et al.*2008.*J cell Sci.*121:2148. (IF) PubMed
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.
13. Banath JP, *et al.* 2010. *BMC Cancer* 10:4 (FC)
14. Zhang M., *et al.* 2011. *Cancer Res.* 23:7155. PubMed.
15. Heo JI, *et al.* 2013. *J Gerontol A Biol Sci Med Sci.* PubMed.

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 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 and immunofluorescence.

BioLegend Inc., 9727 Pacific Heights Blvd, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587

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.

Related Products:

Product	Clone	Application
Cell Staining Buffer		FC, ICC, ICFC
Alexa Fluor® 488 Mouse IgG2b, κ Isotype Ctrl	MPC-11	FC, ICFC

BioLegend Inc., 9727 Pacific Heights Blvd, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587