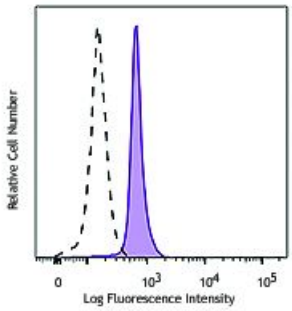
SONY

Product Data Sheet

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

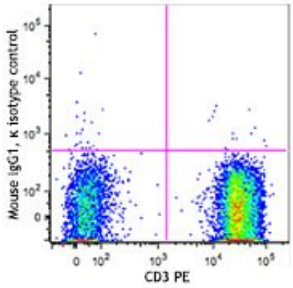
Catalog # / Size:	3667110 / 100 tests 3667105 / 25 tests
Clone:	2F3
Isotype:	Mouse IgG1, κ
Immunogen:	Modified peptide
Reactivity:	Human
Preparation:	The antibody was purified by affinity chromatography and conjugated with PE/Fire™ 700 under optimal conditions.
Formulation:	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide and BSA (origin USA).
Workshop Number:	750 under optimal conditions.
Concentration:	Lot-specific



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).

Applications:

Applications:	Intracellular Staining for Flow Cytometry
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.



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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.

Application References:

1. Jha JC, *et al.* 2013. *J. Virol.* 87:5255. (FC) PubMed
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
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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
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12. Massignan T, *et al.* 2010. *J. Biol Chem.* 285:7752. (WB) PubMed
13. Banath JP, *et al.* 2010. *BMC Cancer* 10:4 (FC)
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15. Kuefner MA, *et al.* 2012. *Radiology* 264:59. PubMed
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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

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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-Martin B, *et al.* 2003. *J. Exp. Med.* 197:1767.

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