SONY

Product Data Sheet

Purified anti-H2A.X Phospho (Ser139)

Catalog # / Size: 3667010 / 100 µg
3667005 / 25 µg

Clone: 2F3

Isotype: Mouse IgG1, κ

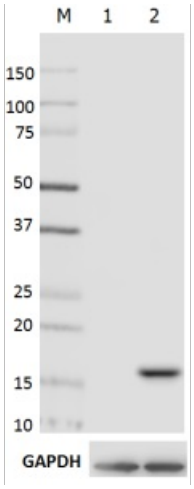
Immunogen: Modified peptide

Reactivity: Human, Other

Preparation: The antibody was purified by affinity chromatography.

Formulation: This H2A.X antibody is provided in phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide. Final antibody concentration is 0.5 mg/ml.

Concentration: 0.5

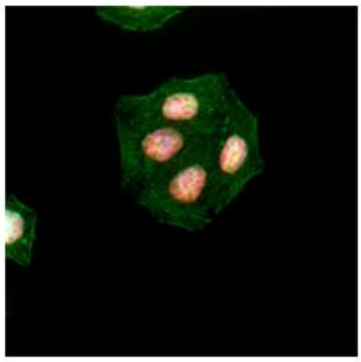


Total lysates (15 µg protein) from untreated HeLa (Lane 1) and UV treated HeLa (Lane 2) cells were resolved by electrophoresis (4-20% Tris-Glycine gel), transferred to nitrocellulose, and probed with 0.1 µg/mL Purified anti-H2A.X Phospho (Ser139) antibody, clone 2F3 (upper) or 1:2000 diluted anti-GAPDH antibody, clone 1D4 (lower). Proteins were visualized by chemiluminescence detection using a 1:3000 diluted goat anti-mouse-IgG secondary antibody conjugated to HRP for both anti-H2A.X Phospho (Ser139) antibody and anti-GAPDH antibody. Lane M: Molecular weight ladder

Applications:

Applications: Immunofluorescence Microscopy, Immunohistochemistry, Other, Intracellular Staining for Flow Cytometry

Recommended Usage: Each lot of this antibody is quality control tested by Western blotting. Western blotting, suggested working dilution(s): Use 5 µg antibody per 5 ml antibody dilution buffer for each mini-gel. For immunofluorescence microscopy: Use a dilution range of 1~4 µg/ml. It is recommended that the reagent be titrated for optimal performance for each application.



Untreated (Lane 1) and staurosporine-treated (Lane 2) Jurkat nuclear extract, untreated (Lane 3) and UV treated (Lane 4) Jurkat cell extract were western blotted using anti-phospho-H2A.X (Ser 139) antibody, clone: 2F3.

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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: 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 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:

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3. Mochizuki K, *et al.*2008.*J cell Sci.*121:2148. (IF) 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.

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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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