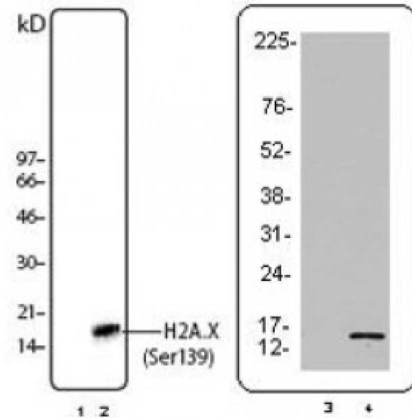
SONY

Product Data Sheet

Purified anti-H2A.X Phospho (Ser139)

Catalog # / Size:	3667005 / 25 µg 3667010 / 100 µg
Clone:	2F3
Isotype:	Mouse IgG1, κ
Immunogen:	Modified peptide
Reactivity:	Human,Mouse
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



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.

Applications:

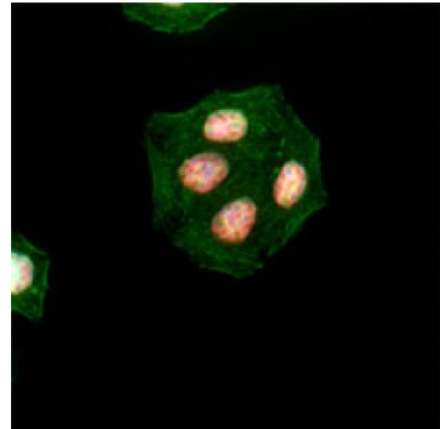
Applications: Other

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

Application Notes: **Additional reported applications (for the relevant formats of this clone) include:** immunohistochemistry on paraffin embedded sections2, immunofluorescence microscopy[3-9], Western blotting [10-12], 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,



HeLa cells were stained with purified anti-H2A.X Phospho (Ser 139) (clone 2F3) antibody, followed by staining with DyLight™ 594 conjugated goat anti-mouse IgG (red) antibody. Actin filaments were labeled in green. Nuclei were stained with DAPI (blue

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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 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.

Antigen References:

1. Manniron 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

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