Phospho-Histone H2A.X Serine 139 Rabbit Polyclonal Antibody

Cat# CY-P1015

25 µg (0.5 mg/ml x 50 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	WB, IF	H, M, R	14 kDa	Rabbit IgG

Background

The histone H2A.X protein is a variant member of the H2A family of histones and is distinguished from other H2A histones by a unique carboxy-terminal sequence. This unique sequence is highly conserved throughout eukaryotic evolution and is rapidly phosphorylated at the fourth residue from the carboxy-terminus (serine 139 in mammalian H2A.X) in response to DNA double-strand breaks (DSBs).

H2A.X phosphorylation is a very rapid response to DNA damage, occurring within as little as one minute after exposure to ionizing radiation. Phosphorylation of H2A.X occurs irrespective of the cause of the DNA DSBs and phospo-H2A.X has been observed in response to environmental stresses that result in DSBs as well as programmed cellular events, including DNA rearrangement and apoptosis.

Specificity/Sensitivity: Phospho-Histone H2A.X Ser139 Antibody detects endogenous Histone H2A.X only when phosphorylated at serine139. The antibody does not recognize other Histone.

Source/Purification: Polyclonal antibody is produced by immunizing rabbits with a synthetic phosphopeptide corresponding to residues surrounding Ser139 of human Histone H2A.X. IgG is purified by phosphopeptide-Sepharose affinity chromatography.

Recommended Antibody Dilutions: Western blotting: 1-2 µg/mL, Immunofluorescence: 2-5 µg/mL

Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key: WB: Western **IP:**Immunoprecipitation **IF:**Immunofluorescence **IHC:**Immunohistochemistry **IC:**Immunocytochemistry **F:**Flow cytometry **E:**ELISA **FP:**Fluorescence Polarization assay
Species Cross-Reactivity Key: H:human **M:**mouse **R:**rat **Hm:**hamster **Mk:**monkey **Mi:**mink **C:**chicken **X:***Xenopus* **Z:**zebra fish **All:**all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Selected Application References:

1. Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ ATM phosphorylates histone H2AX in response to DNA double-strand breaks. *J Biol Chem*. 2001 Nov 9;**276**(45):42462-7.

2. Bassing CH, Suh H, Ferguson DO, Chua KF, Manis J, Eckersdorff M, Gleason M, Bronson R, Lee C, Alt FW. Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. *Cell*. 2003 Aug 8;**114**(3):359-70.

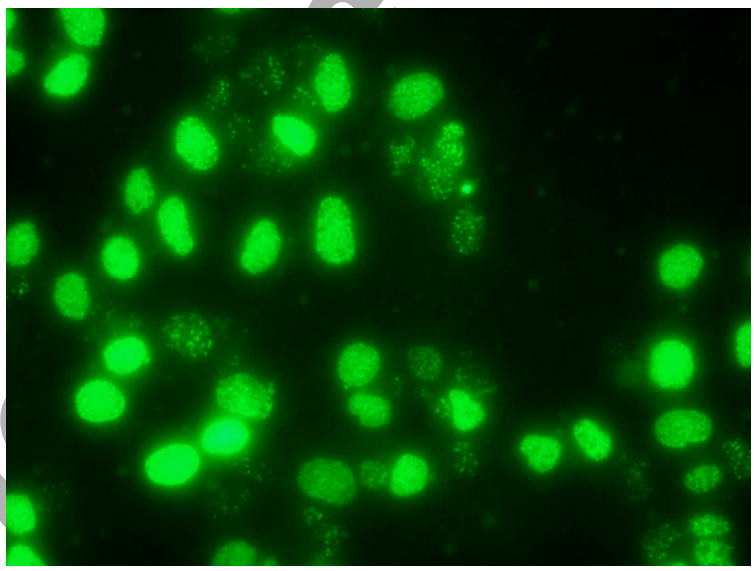
3. Kobayashi J, Tauchi H, Sakamoto S, Nakamura A, Morishima K, Matsuura S, Kobayashi T, Tamai K, Tanimoto K, Komatsu K. NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. *Curr Biol*. 2002 Oct 29;**12**(21):1846-51.

4. Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A. Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. *Nat Cell Biol*. 2003 Jul;**5**(7):675-9.

5. Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM. DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. *J Biol Chem*. 1998 Mar 6;**273**(10):5858-68.

6. Rogakou EP, Nieves-Neira W, Boon C, Pommier Y, Bonner WM. Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. *J Biol Chem*. 2000 Mar 31;**275**(13):9390-5.

Fig. 1 Immunofluorescent staining of MCF7 that has been treated with 10 µM Camptothecin for 30 min using Anti-phospho-Histone H2A.X Serine 139 polyclonal antibody (Cat# CY-P1016)



Western Immunoblotting Protocol

Solutions and Reagents
Note: Prepare solutions with Milli-Q or equivalently purified water.

Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
SDS Sample Buffer (1X): 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
10X TBS (Tris-buffered saline): To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.1% Tween-20 with 5% blocking agent; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Protein Blotting
A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS Sample Buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm2 plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).
8. Electrotransfer to nitrocellulose membrane.

Membrane Blocking and Antibody Incubations
Note: Volumes are for 10 cm x 10 cm (100 cm2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of Blocking Buffer for 1 hour at room temperature.
3. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml Primary Antibody Dilution Buffer with gentle agitation overnight at 4°C.
5. Wash 3 times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:3000 in 10 ml of Blocking Buffer with gentle agitation for 1 hour at room temperature.
7. Wash 3 times for 5 minutes each with 15 ml of TBS/T.

Detection of Proteins

1. Incubate membrane with 4 ml ECL™ with gentle agitation for 1 minute at room temperature.
2. Drain membrane of excess developing solution, do not let dry, wrap in plastic wrap and expose to x-ray film. An initial tensecond exposure should indicate the proper exposure time.

Immunoprecipitation Followed by Western Immunoblotting Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Cell Lysis Buffer (1X): 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Glycerolphosphate, 1 mM Na3VO4, 1 µg/ml Leupeptin
Note: We recommend adding 1 mM PMSF before use.
Protein A Agarose Beads: Add 5 ml of 1X PBS to 1.5 g of Protein A Agarose Beads. Shake 2 hours at 4°C; spin down. Wash pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)
3X SDS Sample Buffer: 187.5 mM Tris-HCl (pH 6.8 at 25°C), 6% w/v SDS, 30%, glycerol, 150 mM DTT, 0.03% w/v bromophenol blue,
Transfer Buffer:25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
Blocking Buffer: 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk. For 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
10X TBS (Tris-buffered saline): For 1 liter of 10X TBS, use 24.2 g Tris base and 80 g NaCl. Adjust pH to 7.6 with HCl (use at 1X).
Primary Antibody Dilution Buffer: 1X TBS, 0.05% Tween-20 with 5% nonfat dry milk. For 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 10 µl Tween-20 (100%).
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Chemiluminescent HRP Detection: secondary anti-rabbit antibody conjugated to horseradish peroxidase (HRP), ECL™ chemiluminescent reagent (Amersham Pharmacia)
Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20
Blotting Membrane: This protocol has been optimized for nitrocellulose membranes, which we recommend. PVDF membranes may also be used.

Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 ml 1X ice-cold Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate 4 times for 5 seconds each on ice.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at –80°C.

Immunoprecipitation

1. Take 200 µl cell lysate and add primary antibody; incubate with gentle rocking overnight at 4°C.
2. Add Protein A Agarose Beads (20 µl of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet 2 times with 500 µl of 1X Cell Lysis Buffer. Keep on ice during washes.
4. Resuspend the pellet with 20 µl 3X SDS Sample Buffer. Votex, then microcentrifuge for 30 seconds.
5. Heat the sample to 95–100°C for 2–5 minutes.
6. Load the sample (15–30 µl) on SDS-PAGE gel (12–15%).

7. Analyze sample by Western blotting (see Western Immunoblotting Protocol).

Immunofluorescence analysis protocol

1. Grow cells to a density of $1X10^6$ cells/ml.
2. Treat cells with 0.5_M staurosporine (Catalog # 19-123) for 4 to 6 hours.
3. Collect cells by centrifugation at 1200 RPM for 5 minutes. Decant media.
4. Wash cells one time with TBS
5. Add fixing agent 95% ethanol 5% acetic acid for five minutes.
6. Centrifuge and decant, add blocking buffer 3% BSA TBS and block for 30 minutes.
7. Centrifuge and decant add 2_g/ml of anti-phospho-Histone H2A.X (Ser139) in blocking buffer for 1 hour.
8. Centrifuge and decant, wash with TBS for 5 minutes.
9. Centrifuge and decant, add secondary antibody in blocking buffer (2_g/ml of goat anti-mouse Oregon GreenTM from Molecular Probes was used). Incubate in the dark, 1-1.5 hours.
10. Wash 5 times with TBS.
11. Mount cells on slides utilizing cytospin apparatus or your preferred technique. Observe cells under light of wavelength which is appropriate for the fluorescent secondary conjugate.

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