



cell signaling solutions

Certificate of Analysis

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Anti-phospho-H2A.X (Ser139)

(rabbit polyclonal IgG)

Catalog # 07-164

Lot # 23646

Immunogen: KLH-conjugated, synthetic peptide (C-KATQA[pS]QEY) corresponding to amino acids 134-142 of human histone H2A.X. The immunizing sequence has 8 identical amino acids in yeast and mouse.

Specificity: Recognizes Histone H2A phosphorylated at Ser139, Mr 14kDa.

Species Cross-reactivity: Human; broad species cross-reactivity expected based on conservation of sequence homology.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

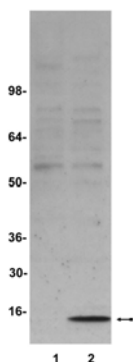
Formulation: 200µg of protein A purified rabbit IgG in 200µl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.1-1µg/ml of this lot detected phosphorylated histone H2A.X (Ser139) in RIPA lysates from Jurkat cells treated with staurosporine (Catalog # 19-123).

0.1-1µg/ml of a previous lot detected phosphorylated histone H2A.X (Ser139) in acid-extracted proteins from γ-irradiated human astrocytoma SF268 cells.



Immunoblot Analysis

Representative blot from a previous lot. Untreated (lane 1), and staurosporine-treated (lane 2) Jurkat cells lysates were resolved by electrophoresis, transferred to nitrocellulose and probed with anti-phospho H2A.X (Ser139) (1µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates phosphorylated H2A.X (14kDa).

Additional Research Applications

Immunocytochemistry: As shown by an independent laboratory, this antibody detected phosphorylated H2A.X in IMR90, MCF7, Mo59J, and NBS cells irradiated with a ¹³⁷Cs source in a Mark I irradiator and allowed to recover at 37°C for various amounts of time.

Immunoprecipitation: This antibody successfully immunoprecipitates phosphorylated H2A.X as determined by an independent laboratory.

Application Reference:

Paull, T.T., *et al.*, *Current Bio.* **10**: 886-895, 2000.

Rogakou, E., *et al.*, *J Cell Biol.* **146**: 905-916, 1999.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a RIPA lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared 3% nonfat dry milk (Catalog # 20-200) in TBS (TBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.1-1µg/ml of anti-phospho-H2A.X (Ser139)**, diluted in freshly prepared TBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in TBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

Acid Extraction of Proteins from Cells

1. Grow cells to 70% confluency in DMEM supplemented with 10% FBS.
2. Add sodium butyrate (100mM sterile stock solution), which inhibits histone deacetylases, to a final concentration of 5mM and continue to grow the cells for 24 hours.
3. Scrape the cells from the plate.
4. Pellet the cells by centrifugation at 200 x g for 10 minutes.
5. Decant the supernatant fraction.
6. Suspend the cells with 10-15 volumes of PBS and centrifuge at 200 x g for 10 minutes.
7. Decant supernatant fraction (PBS wash).
8. Suspend the cell pellet in 5-10 volumes of lysis buffer*.
9. Add sulfuric acid to a final concentration of 0.2M (0.4N). **Use polypropylene tubes.**
10. Incubate on ice for 30 minutes.
11. Centrifuge at 11,000 x g for 10 minutes at 4°C.
12. Keep the supernatant fraction, which contains the acid soluble proteins, and discard the acid-insoluble pellet.
13. Dialyze the supernatant against 200ml 0.1M (0.1N) acetic acid, twice for 1-2 hours each.
14. Dialyze three times against 200ml H₂O for 1hour, 3 hours, and overnight, respectively. The protein can be quantified and lyophilized or stored at -70°C.

*Lysis buffer:

10mM HEPES, pH 7.9
1.5mM MgCl₂
10mM KCl
*0.5mM DTT
*1.5mM PMSF

*Add PMSF and DTT just prior to use of the buffer.