

# Anti-phospho-H2A.X (Ser139)

Polyclonal Antibody

Cat. # 07-164

Lot # DAM1546024

pack size: 200 µg

Store at -20°C

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS



## Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP	H, WR	IgG	a.a. 134-142	Rb	15 kDa	NP002096

### Background

Histone H2A is one of the 5 main histone proteins involved in the structure of chromatin in eukaryotic cells. Featuring a main globular domain and a long N terminal tail H2A is involved with the structure of the nucleosomes of the 'beads on a string' structure.

### Presentation

Purified rabbit polyclonal IgG in buffer containing 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide.

### Concentration

1 mg/mL

### Specificity

Histone H2A.X phosphorylated at Ser139.

### Species Cross-reactivity

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

### Immunogen

KLH-conjugated, synthetic peptide (CKATQA[*p*S]QEY) corresponding to amino acids 134-142 of human histone H2A.X. The immunizing sequence has 8 identical amino acids in yeast and mouse.

### Molecular Weight

15 kDa

### Method of Purification

Protein A chromatography

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon receipt, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C.

Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance. Note: Variability in freezer temperatures below -20°C may cause glycerol-containing solutions to become frozen during storage.

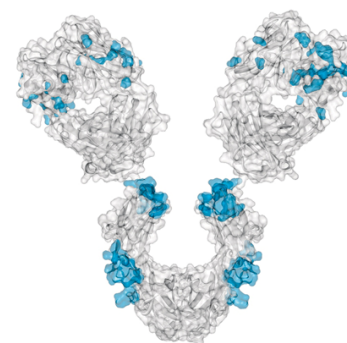
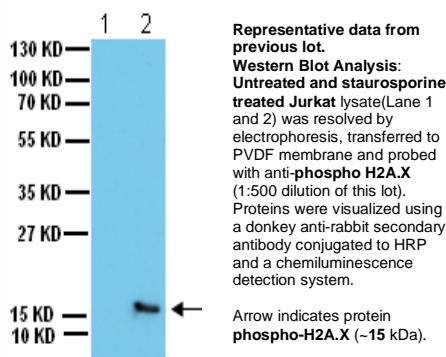
### Control

Staurosporine-treated Jurkat lysate, UV-treated HT-29 cells, doxorubicin-treated HT-29 cells.

### Quality Control Testing

Routinely evaluated by Western Blot on untreated and staurosporine treated Jurkat lysate.

**Western Blot Analysis:** 1:500 dilution of this lot detected **phospho-H2A.X** on 10 µg of **staurosporine treated Jurkat lysate**.



### References

1. Chowdhury, D., *et al.* (2005). *Mol. Cell.* 20: 801-809.
2. Yih, L. H., *et al.* (2005). *Carcinogenesis.* 26: 53-63.
3. d'Adda di Fagagna, F., *et al.* (2003). *Nature.* 426: 194-8.
4. Hamer, G., *et al.* (2003). *Biol Reprod.* 68: 628-34.
5. Li, J., *et al.* (2003). *J Biol Chem.* 278: 13183-91.
6. Paull, T. T., *et al.* (2000). *Current Bio.* 10: 886-895.

**Immunoprecipitation:** A previous lot of this antibody successfully immunoprecipitated phosphorylated H2A.X as determined by an independent laboratory.

### Storage and Handling

### Additional Research Applications

**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence IH Immunohistochemistry (Tissue)

**SPECIES LEGEND:** H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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**PROTOCOL****Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a RIPA lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na3VO4; 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared TBS containing 3% nonfat dry milk (Catalog # 20-200), (TBS-MLK) for 20 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.1-1 µg/mL of anti-phospho-H2A.X (Ser139)**, diluted in freshly prepared TBSMLK and 0.1% Tween 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 and 0.1% Tween for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in TBS-MLK and 0.1% 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 (100 mM sterile stock solution), which inhibits histone deacetylases, to a final concentration of 5 mM 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 0.2 M (0.4 N). **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 200 mL 0.1 M (0.1 N) acetic acid, twice for 1-2 hours each.
14. Dialyze three times against 200 mL H2O for 1 hour, 3 hours, and overnight, respectively. The protein can be quantified and lyophilized or stored at -70°C.

**\*Lysis buffer:**

10 mM HEPES, pH 7.9	*0.5 mM DTT
1.5 mM MgCl <sub>2</sub>	*1.5 mM PMSF
10 mM KCl	

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

**RELATED PRODUCTS (specific)**

cat #	description
07-627	■ Anti-Histone H2A.X
05-636	■ Anti-phospho-Histone H2A.X (Ser139), clone JBW301
16-193	■ Anti-phospho-Histone H2A.X (Ser139), clone JBW301, biotin conjugate
16-202A	■ Anti-phospho-Histone H2A.X (Ser139), FITC conjugate
14-576	■ Histone H2A.X
17-327	■ H2A.X Phosphorylation Assay Kit (Chemiluminescence Detection)
17-344	■ H2A.X Phosphorylation Assay Kit (Flow Cytometry)
17-306	■ Control Histones (+/- colcemid)
12-348	■ Goat Anti-Rabbit IgG

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm IPVH07850
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 µm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 µm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 µm (sheet) 10/pk
WBKLS0050	■ IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	■ Spray & Glow™ ECL Western Blotting 40 mL
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
2170	■ CHEMIBLOCKER-1LT
20-200	■ WESTERN BLOT BLOCKING REAGENT 20G
12-302	■ EGF-Stimulated A431 Cell Lysate
12-349	■ Goat Anti-Mouse IgG, HRP conjugate
12-110	■ Phosphotyrosine control (EGF-stim A431 cell lysate)

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