

Store at  
4°C

#50929

# PathScan® Phospho-Histone H2A.X (Ser139) Sandwich ELISA Kit

1 Kit  
(96 assays)



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New 04/18

For Research Use Only. Not For Use In Diagnostic Procedures.

## Species Cross-Reactivity: H

**Description:** The PathScan® Phospho-Histone H2A.X (Ser139) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Histone H2A.X protein phosphorylated at Ser139. A Histone H2A.X rabbit antibody has been coated onto the microwells. After incubation with cell lysates, both phospho- and non-phospho-Histone H2A.X proteins are captured by the coated antibody. Following extensive washing, a phospho-Histone H2A.X (Ser139) mouse antibody is added to detect the captured phospho-Histone H2A.X protein. Anti-mouse IgG, HRP-linked Antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of Histone H2A.X phosphorylated at Ser139. Antibodies in this kit are custom formulations specific to kit.

**Background:** Histone H2A.X is a variant histone that represents approximately 10% of the total H2A histone proteins in normal human fibroblasts (1). H2A.X is required for checkpoint-mediated cell cycle arrest and DNA repair following double-stranded DNA breaks (1). DNA damage, caused by ionizing radiation, UV-light, or radiomimetic agents, results in rapid phosphorylation of H2A.X at Ser139 by PI3K-like kinases, including ATM, ATR, and DNA-PK (2,3). Within minutes following DNA damage, H2A.X is phosphorylated at Ser139 at sites of DNA damage (4). This very early event in the DNA-damage response is required for recruitment of a multitude of DNA-damage response proteins, including MDC1, NBS1, RAD50, MRE11, 53BP1, and BRCA1 (1). In addition to its role in DNA-damage repair, H2A.X is required for DNA fragmentation during apoptosis and is phosphorylated by various kinases in response to apoptotic signals. H2A.X is phosphorylated at Ser139 by DNA-PK in response to cell death receptor activation, c-Jun N-terminal Kinase (JNK1) in response to UV-A irradiation, and p38 MAPK in response to serum starvation (5-8). H2A.X is constitutively phosphorylated on Tyr142 in undamaged cells by WSTF (Williams-Beuren syndrome transcription factor) (9,10). Upon DNA damage, and concurrent with phosphorylation of Ser139, Tyr142 is dephosphorylated at sites of DNA damage by recruited EYA1 and EYA3 phosphatases (9). While phosphorylation at Ser139 facilitates the recruitment of DNA repair proteins and apoptotic proteins to sites of DNA damage, phosphorylation at Tyr142 appears to determine which set of proteins are recruited. Phosphorylation of H2A.X at Tyr142 inhibits the recruitment of DNA repair proteins and promotes binding of pro-apoptotic factors such as JNK1 (9). Mouse embryonic fibroblasts expressing only mutant H2A.X Y142F, which favors recruitment of DNA repair proteins over apoptotic proteins, show a reduced apoptotic response to ionizing radiation (9). Thus, it appears that the balance of H2A.X Tyr142 phosphorylation and dephosphorylation provides a switch mechanism to determine cell fate after DNA damage.

Products Included	Item #	Kit Quantity	Color	Storage Temp
H2A.X Rabbit mAb Coated Microwells*	17725	96 tests		4°C
P-Histone H2A.X (Ser139) Mouse Detection mAb	49027	1 each	Green (Lyophilized)	4°C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

\* 12 8-well modules -Each module is designed to break apart for 8 tests.

**Specificity/Sensitivity:** PathScan® Phospho-Histone H2A.X (Ser139) Sandwich ELISA Kit detects endogenous levels of Histone H2A.X protein phosphorylated at Ser139 in human cells, as shown in Figure 1. The kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

**Storage:** Kit should be stored at 4°C with the exception of Cell Lysis Buffer, which is stored at -20°C (packaged separately).

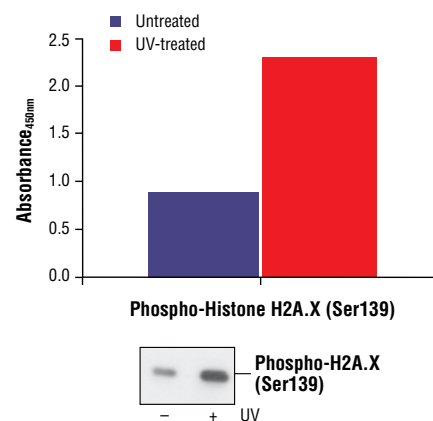


Figure 1. Treatment of 293 cells with UV light stimulates phosphorylation of Histone H2A.X at Ser139, detected by the PathScan® Phospho-Histone H2A.X (Ser139) Sandwich ELISA Kit. In contrast, a lower signal of phospho-Histone H2A.X protein is seen untreated lysate. The levels of total Histone H2A.X protein from both untreated and treated lysates are similar by western analysis (data not shown). The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blot using Phospho-Histone H2A.X (Ser139) (20E3) Rabbit mAb #9718 is shown in the bottom figure.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

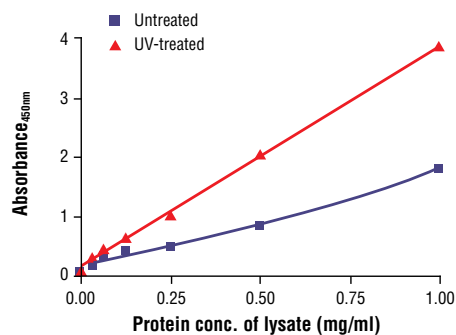


Figure 2: The relationship between protein concentration of lysates from untreated and UV-treated 293 cells and the absorbance at 450 nm as detected by the PathScan® Phospho-Histone H2A.X Ser139 Sandwich ELISA Kit is shown. Unstarved 293 cells (80-90% confluence) were treated with 100 mJ/cm<sup>2</sup> UV with 1 hr recovery at 37°C, and then lysed with Cell Lysis Buffer (#9803).

#### Background References:

- (1) Yuan, J. et al. (2010) *FEBS Lett* 584, 3717-24.
- (2) Rogakou, E.P. et al. (1998) *J Biol Chem* 273, 5858-68.
- (3) Burma, S. et al. (2001) *J Biol Chem* 276, 42462-7.
- (4) Rogakou, E.P. et al. (1999) *J Cell Biol* 146, 905-16.
- (5) Mukherjee, B. et al. (2006) *DNA Repair (Amst)* 5, 575-90.
- (6) Solier, S. et al. (2009) *Mol Cell Biol* 29, 68-82.
- (7) Lu, C. et al. (2006) *Mol Cell* 23, 121-32.
- (8) Lu, C. et al. (2008) *FEBS Lett* 582, 2703-8.
- (9) Cook, P.J. et al. (2009) *Nature* 458, 591-6.
- (10) Xiao, A. et al. (2009) *Nature* 457, 57-62.

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## PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

### A Solutions and Reagents

**NOTE:** Prepare solutions with purified water.

- 1. Microwell strips:** Bring all to room temperature before use.
- 2. Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- 3. HRP-Linked Antibody\*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- 4. Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- 5. HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- 6. Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 7. 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 8. Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethyl-sulfonyl fluoride (PMSF) immediately before use.
- 9. TMB Substrate (#7004).**
- 10. STOP Solution (#7002).**

*\*Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.*

### B Preparing Cell Lysates

#### For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

#### For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 × 10<sup>6</sup> viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

### C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
  - a. Discard plate contents into a receptacle.
  - b. Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
  - c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
  - d. Clean the underside of all wells with a lint-free tissue.
5. Add 100 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
10. Add 100 µl of STOP Solution to each well. Shake gently for a few seconds.

**NOTE:** Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
  - a. **Visual Determination:** Read within 30 min after adding STOP Solution.
  - b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.