#7870C Store at 4°C

PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit

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☑ 1 Kit
(96 assays)

rev. 08/01/17

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

Species Cross-Reactivity: H

Description: The PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Chk1 when phosphorylated at Ser317. An Chk1 Mouse Antibody* has been coated onto the microwells. After incubation with cell lysates, Chk1 (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Phospho-Chk1 (Ser317) Rabbit Detection Antibody* is added to detect phosphorylation of Ser317 on the captured Chk1 protein. Anti-rabbit IgG, HRP-linked Antibody #7074* 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 Chk1 phosphorylated at Ser317.

*Antibodies in kit are custom formulations specific to kit.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
Chk1 Mouse mAb Coated Microwells*	19914	96 tests		4°C
Phospho-Chk1 (Ser317) Detection Antibody	14014	1 each	Green (Lyophilized)	4°C
Anti-rabbit IgG, HRP-linked Antibody (ELISA Formulated)	13272	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: CST's PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit #7870 detects endogenous levels of Chk1 protein when phosphorylated at Ser317. As shown in Figure 1, a significant induction of Chk1 phosphorylation at Ser317 can be detected in HeLa cells following treatment with UV using the Phospho-Chk1 (Ser317) Sandwich ELISA Kit #7870. The levels of total Chk1 (phospho and nonphospho) remain unchanged as shown by Western analysis and by PathScan® Total Chk1 Sandwich ELISA Kit #7872 (Figure 1).

Background: Chk1 kinase acts downstream of ATM/ATR kinase to play an important role in DNA damage checkpoint control, embryonic development and tumor suppression (1). Activation of Chk1 involves phosphorylation of Ser317 and Ser345 and occurs in response to blocked DNA replication and certain forms of genotoxic stress (2). Chk1 is also phosphorylated at Ser280 and Ser296 following DNA damage. Activated Chk1 can inactivate cdc25C via phosphorylation at Ser216, blocking the activation of cdc2 and transition into mitosis (3). Chk1 can also phosphorylate p53 at Ser20 *in vitro* (4).

Background References:

(1) Martinho, R.G. et al. (1998) *EMBO J.* 17, 7239–7249.
(2) Zhao, H. et al. (2001) *Mol. Cell. Biol.* 21, 4129–4139.
(3) Zeng, Y. et al. (1998) *Nature* 395, 507–510.
(4) Shieh, S. et al. (2000) *Genes Dev.* 14, 289–300.

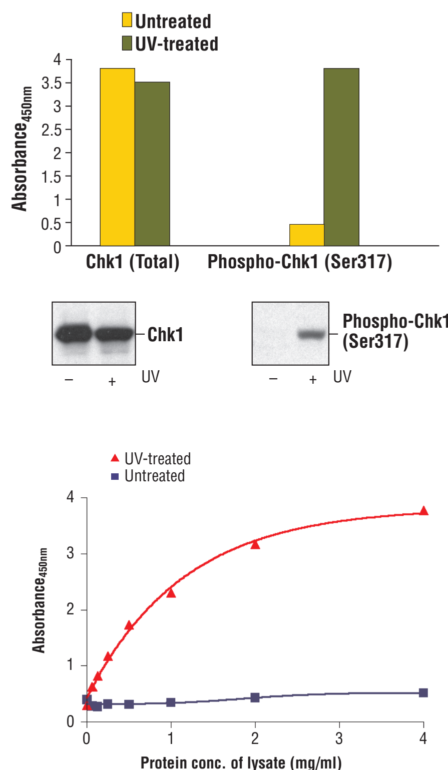

Figure 1. Treatment of HeLa cells with UV stimulates phosphorylation of Chk1 at Ser317, detected by the PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit #7870, but does not affect the levels of total Chk1 detected by PathScan® Total Chk1 Sandwich ELISA Kit #7872. HeLa cells (80-90% confluent) were treated with 100 mJ/cm² UV with 1 hour recovery at 37°C. The absorbance readings at 450 nm are shown in the top figure, while the corresponding Western blots using Chk1 Antibody #2345 (left panel) or Phospho-Chk1 (Ser317) Antibody #2344 (right panel) are shown in the bottom figure.

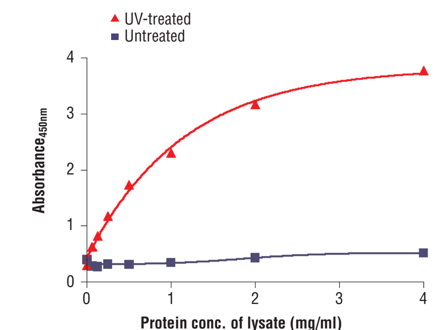

Figure 2. The relationship between the protein concentration of lysates from untreated and UV-treated HeLa cells and the absorbance at 450 nm using the PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit #7870 is shown.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **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 **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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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 or 1X Cell Lysis Buffer #7018: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl 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 x 10^6 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.

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