Store at 4°C

#7872

PathScan® Total Chk1 Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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

Species Cross-Reactivity: H

Description: The PathScan® Chk1 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Chk1. A 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 Chk1 Rabbit Detection Antibody is added to the captured phospho and nonphospho 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 total Chk1.

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

Specificity/Sensitivity: CST's PathScan® Chk1 Sandwich ELISA Kit #7872 detects endogenous levels of total Chk1 protein. 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.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
Chk1 Mouse mAb Coated Microwells*	32550	96 tests		4°C
Chk1 Detection Antibody	37897	11 ml	Green	4°C
Anti-rabbit IgG, HRP-linked Antibody	93699	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.

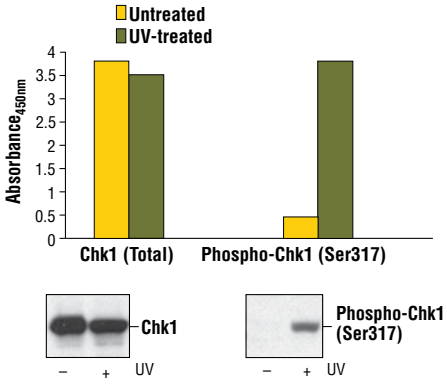

Figure 1. Treatment of HeLa cells with UV stimulates phosphorylation of Chk1 at Ser317, detected by the PathScan® Phospho-Chk1 (Ser317) Sandwich ELISA Kit #7870, but does not affect the levels of total Chk1 detected by PathScan® Total Chk1 Sandwich ELISA Kit #7872. HeLa cells (80-90% confluent) were 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.

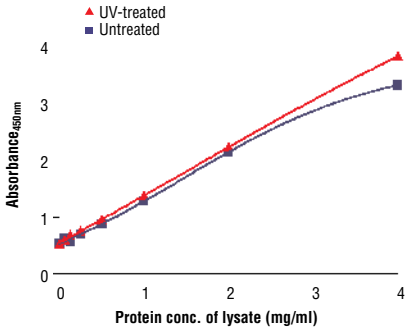

Figure 2. The relationship between the protein concentration of lysates from untreated and UV-treated HeLa cells and the absorbance at 450 nm using the PathScan® Total Chk1 Sandwich ELISA Kit #7872 is shown.

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**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

Sandwich ELISA Protocol

A Reagent Preparation

1. Bring all microwell strips to room temperature before use.
2. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in Milli-Q or equivalently purified water.
3. **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH 7.5), 150 mM NaCl, 1 mM ethylene diamine tetraacetate (EDTA), 1 mM ethylene glycol-bis(2-aminoethyl)-N,N,N',N'-tetraacetic acid (EGTA), 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1µg/ml leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B 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 ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm in diameter) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 minutes 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. Add 100 µl of Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color) to a microcentrifuge tube. Transfer 100 µl of cell lysate into the tube and vortex for a few seconds. Generally, sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction. Individual data sheets for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results. However, dilution factors need to be titrated when specific cell lysates are used.
3. Add 100 µl of each diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hours at 37°C. Alternatively, the plate can be incubated overnight at 4°C, which gives the best detection of target protein.
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 Detection Antibody (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.
6. Repeat wash procedure as in Step 4.
7. Add 100 µl of HRP-linked secondary antibody (red color) to each well. Seal with tape and incubate the plate for 30 minutes at 37°C.
8. Repeat wash procedure as in Step 4.
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 minutes at 37°C or 30 minutes 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 minutes after adding STOP Solution.
    b. Spectrophotometric Determination — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.