

PathScan® Total Chk2 Sandwich ELISA Kit

✓ 1 Kit
(96 assays)

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New 10/09

This product is for *in vitro* research use only and is not intended for use in humans or animals.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID #6850
Swiss-Prot Acc. #P43405

Species Cross-Reactivity: H

Description: The PathScan® Total Chk2 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of Chk2. A Chk2 rabbit antibody has been coated onto the microwells. After incubation with cell lysates, Chk2 (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Chk2 mouse detection antibody is added to the captured phospho and nonphospho Chk2 protein. Anti-mouse IgG, HRP-linked Antibody #7076 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 Chk2.

Specificity/Sensitivity: CST's PathScan® Chk2 Sandwich ELISA Kit #7045 detects endogenous levels of total Chk2 protein (see Figure 1). The kit sensitivity is shown in Figure 2.

Background: Chk2 is the mammalian orthologue of the budding yeast Rad53 and fission yeast Cds1 checkpoint kinases (1-3). The amino-terminal domain of Chk2 contains a series of seven serine or threonine residues (Ser19, Thr26, Ser28, Ser33, Ser35, Ser50 and Thr68) each followed by glutamine (SQ or TQ motif). These are known to be preferred sites for phosphorylation by ATM/ATR kinases (4,5). After DNA damage by ionizing radiation (IR), UV irradiation or hydroxyurea treatment, Thr68 and other sites in this region become phosphorylated by ATM/ATR (5-7). The SQ/TQ cluster domain, therefore, seems to have a regulatory function. Phosphorylation at Thr68 is a prerequisite for the subsequent activation step, which is attributable to auto-phosphorylation of Chk2 on residues Thr383 and Thr387 in the activation loop of the kinase domain (8).

Background References:

- (1) Allen, J.B. et al. (1994) *Genes Dev.* 8, 2401-2415.
- (2) Weinert, T.A. et al. (1994) *Genes Dev.* 8, 652-665.
- (3) Murakami, H. and Okayama, H. (1995) *Nature* 374, 817-819.
- (4) Kastan, M.B. and Lim, D.S. (2000) *Nat. Rev. Mol. Cell Biol.* 1, 179-186.
- (5) Matsuoka, S. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 10389-10394.
- (6) Melchionna, R. et al. (2000) *Nat. Cell Biol.* 2, 762-765.
- (7) Ahn, J.Y. et al. (2000) *Cancer Res.* 60, 5934-5936.
- (8) Lee, C.H. and Chung, J.H. (2001) *J. Biol. Chem.* 276, 30537-30541.

Products Included	Volume	Solution Color
Chk2 Rabbit Antibody Coated Microwells*	96 tests	
Chk2 Mouse Detection Antibody	11 ml	Green
Anti-mouse IgG, HRP-linked Antibody	11 ml	Red
TMB Substrate	11 ml	Colorless
STOP Solution	11 ml	Colorless
Sealing Tape	2 sheets	
20X Wash Buffer	25 ml	Colorless
Sample Diluent	25 ml	Blue
10X Cell Lysis Buffer #9803**	15 ml	Yellowish

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

**Kit should be stored at 4°C with the exception of 10X Cell Lysis Buffer, which is stored at -20°C (packaged separately).

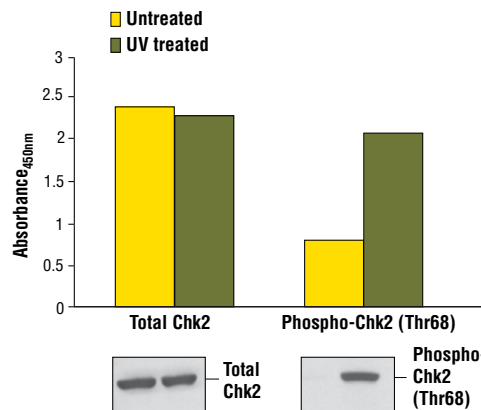


Figure 1. Treatment of HeLa cells with UV stimulates phosphorylation of Chk2 at Thr68, detected by the PathScan® Phospho-Chk2 (Thr68) Sandwich ELISA Kit #7037, but does not affect the levels of total Chk2 detected by PathScan® Total Chk2 Sandwich ELISA Kit #7045. HeLa cells (80-90% confluent) were treated 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 Chk2 (1C12) Mouse mAb #3440 (left panel) or Phospho-Chk2 (Thr68) (C13C1) RmAb #2197 (right panel) are shown in the bottom figure.

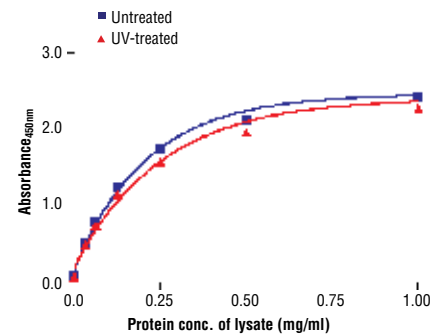


Figure 2. The relationship between the protein concentration of lysates from untreated and UV-treated HeLa cells and the absorbance at 450 nm using the PathScan® Total Chk2 Sandwich ELISA Kit #7045 is shown.

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 Na₃VO₄, 1 μg/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 phenyl-methylsulfonyl 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.