

PathScan® Total p53 Sandwich ELISA Kit

✓ 1 Kit (96 assays)

new 08/04



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Species Cross-Reactivity: H

Introduction: CST's PathScan® Total p53 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total p53 protein. A p53 Rabbit mAb (7365-15A5)* has been coated onto the microwells. After incubation with cell lysates, Both nonphospho- and phospho-p53 proteins are captured by the coated antibody. Following extensive washing, a p53 (1C12) Mouse mAb #2524* is added to detect the captured p53 protein. HRP-linked anti-mouse antibody #7076* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of total p53 protein.

*Antibodies in this kit are custom formulations specific to the kit.

Companion Products:

PathScan® Phospho-p53 (Ser15) Sandwich ELISA Kit #7365

p53 (1C12) Mouse mAb #2524

Phospho-p53 (Ser15) (16G8) Mouse mAb #9286

Anti-mouse IgG, HRP-linked Antibody #7076

Cell Lysis Buffer (10X) #9803

Specificity/Sensitivity: CST's PathScan® Total p53 Sandwich ELISA Kit detects endogenous levels of total p53 protein. Using PathScan® Phospho-p53 (Ser15) Sandwich ELISA Kit #7365, a significant induction of phospho-p53 in HT-29 cells treated with UV can be detected. However, the level of total p53 (phospho and non-phospho), detected by this Sandwich ELISA Kit #7370, remains unchanged (Figure 1).

Kit Includes

Products	Volume	Solution Color
p53 (7365-15A5) rabbit mAb Coated Microwells *	96 tests	
p53 Detection Ab	11 ml	green
Anti-mouse IgG HRP-Linked Ab	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).

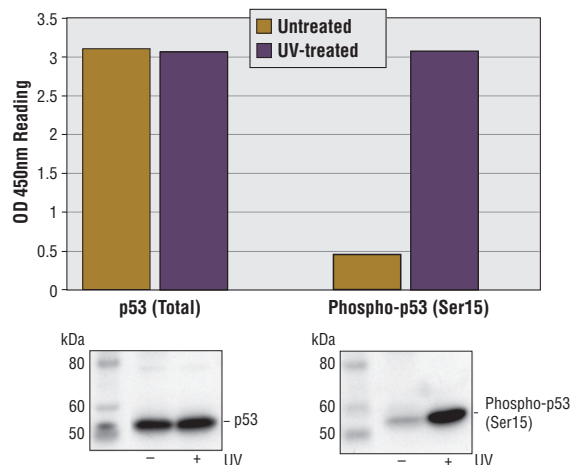


Figure 1: Treatment of HT-29 cells with UV stimulates phosphorylation of p53 at Ser15, detected by PathScan® Phospho-p53 (Ser15) Sandwich ELISA kit, #7365, but does not affect the level of total p53 protein detected by PathScan® Total p53 Sandwich ELISA kit, #7370. OD450 readings are shown in the top figure, while the corresponding Western blot using Phospho-p53 (Ser15) Mouse mAb #9286 (right panel) or p53 Mouse mAb #2524 (left panel), is shown in the bottom figure.

Background: The p53 tumor suppressor protein plays a major role in cellular response to DNA damage and other genomic aberrations. Activation of p53 can lead to either cell cycle arrest and DNA repair or apoptosis (1). p53 is phosphorylated at multiple sites *in vivo* and by several different protein kinases *in vitro* (2,3). DNA damage induces phosphorylation of p53 at Ser15 and Ser20 and leads to reduced interaction of p53 with its negative regulator, oncoprotein MDM2 (4). MDM2 inhibits the accumulation of p53 by targeting it for ubiquitination and proteasomal degradation (6,7).

p53 can apparently be phosphorylated by ATM, ATR and DNA-PK at Ser15 and Ser37; the phosphorylations impair the ability of MDM2 to bind p53, promoting both the accumulation and functional activation of p53 in response to DNA damage (4,5). Chk2 and Chk1 can phosphorylate p53 at Ser20, enhancing its tetramerization, stability and activity (8,9). p53 is phosphorylated at Ser392 *in vivo* (11,12) and by CAK *in vitro* (12). Phosphorylation of p53 at Ser392 is altered in human tumors (14) and has been reported to influence the growth suppressor function, DNA binding and transcriptional activation of p53 (10,11,13). p53 is phosphorylated at Ser6 and Ser9 by ck1 δ and ck1 ϵ both *in vitro* and *in vivo* (10,15). Phosphorylation of p53 at Ser46 is important in regulating the ability of p53 to induce apoptosis (16).

Background References:

- (1) Levine, A.J. (1997) *Cell* 88, 323–331.
- (2) Meek, D.W. (1994) *Semin. Cancer Biol.* 5, 203–210.
- (3) Milczarek, G.J. et al. (1997) *Life Sci.* 60, 1–11.
- (4) Shieh, S.Y. et al. (1997) *Cell* 91, 325–334.
- (5) Tibbetts, R.S. et al. (1999) *Genes Dev.* 13, 152–157.
- (6) Chehab, N.H. et al. (1999) *Proc. Natl. Acad. Sci. USA* 96, 13777–13782.
- (7) Honda, R. et al. (1997) *FEBS Lett.* 420, 25–27.
- (8) Shieh, S.Y. et al. (1999) *EMBO J.* 18, 1815–1823.
- (9) Hirao, A. et al. (2000) *Science* 287, 1824–1827.
- (10) Kohn, K.W. (1999) *Mol. Biol. Cell* 10, 2703–2734.
- (11) Hao, M. et al. (1996) *J. Biol. Chem.* 271, 29380–29385.
- (12) Lu, H. et al. (1997) *Mol. Cell. Biol.* 17, 5923–5934.
- (13) Lohrum, M. and Scheidtmann, K.H. (1996) *Oncogene* 13, 2527–2539.
- (14) Ulrich, S.J. et al. (1993) *Proc. Natl. Acad. Sci. USA* 90, 5954–5958.
- (15) Knippschild, U. et al. (1997) *Oncogene* 15, 1727–1736.
- (16) Oda, K. et al. (2000) *Cell* 102, 849–862.

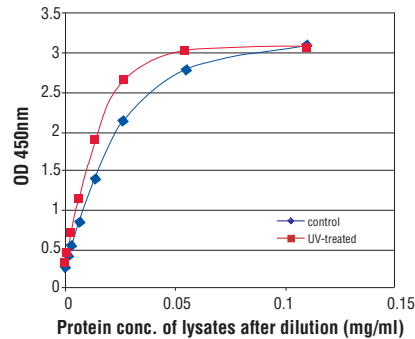


Figure 2: Linear relationship between protein concentration of lysates from UV-treated and control HT-29 cells and kit assay optical density readings. HT-29 cells (80% confluence) were UV-treated and lysed after incubation at 37°C for 2 hours.

Sandwich ELISA Protocol

A Reagent Preparation

- A1** Bring all microwell strips to room temperature before use.
- A2** Prepare 1X Wash Buffer using Milli-Q or equivalently purified water.
- A3** **1X Cell Lysis Buffer from CST #9803:** 20 mM Tris (pH7.5), 150 mM NaCl, 1 mM ethylene diaminetetraacetate (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 -glycerolphosphate, 1 mM Na_3VO_4 , 1 $\mu\text{g}/\text{ml}$ leupeptin. This buffer can be stored at 4°C for short-term use (1–2 weeks).

B Preparing Cell Lysates

- B1** Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
- B2** To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- B3** Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm^2) and incubate the plate on ice for 5 minutes.
- B4** Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- B5** Sonicate lysates on ice.
- B6** Microcentrifuge for 10 minutes at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Aliquot and store at –80°C.

C Test Procedure

- C1** After the microwell strips have reached room temperature, break off the required numbered of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
- C2** Add 100 μl of **Sample Diluent** (blue color) to a microcentrifuge tube. Transfer 100 μl of cell lysate into the tube and vortex for a few seconds. (Sample applied to the well can be diluted 1:1 when the suggested cell lysis buffer is used for cell extraction.) Figure 2 provides a reference in dilution factor for lysates and kit assay results.
- C3** 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.

C4 Gently remove the tape and wash wells:

- Discard plate contents into a receptacle.
- Wash 4 times with 1X Wash Buffer, 200 μl each time for each well.
- 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.
- Clean the underside of all wells with a lint-free tissue.

C5 Add 100 μl of **Detection Antibody** (green color) to each well. Seal with tape and incubate the plate for 1 hour at 37°C.

C6 Repeat wash procedure as in Step C4.

C7 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.

C8 Repeat wash procedure as in Step C4.

C9 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.

C10 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.

C11 Read results.

- Visual Determination** — Read within 30 minutes after adding STOP Solution.
- Spectrophometric Determination** — Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 minutes after adding STOP Solution.