

PathScan® Acetylated p53 Sandwich ELISA Kit



Cell Signaling
TECHNOLOGY®

✓ 1 Kit
(96 assays)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

Species Cross-Reactivity: H, M, Mk

Introduction: The PathScan® Acetylated p53 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of acetylated lysines on p53. A p53 Rabbit Antibody* has been coated onto the microwells. After incubation with cell lysates, the p53 is captured by the coated antibody. Following extensive washing, Acetylated-Lysine Mouse Antibody* is added to detect the acetylated lysines on the p53 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. HRP substrate, TMB is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of acetylated p53.

* Antibodies in kit are custom formulations specific to kit.

Companion Products:

PathScan® Acetylated p53 Sandwich ELISA Kit #7236

Acetylated-Lysine Antibody #9441

Acetylated-Lysine Mouse mAb (Ac-K-103) #9681

p53 Antibody #9282

p53 (7F5) Rabbit mAb #2527

p53 (1C12) Mouse mAb #2524

PathScan® Acetylated Histone H2A Sandwich ELISA Kit #7233

PathScan® Acetylated Histone H3 Sandwich ELISA Kit #7232

PathScan® Acetylated Histone H4 Sandwich ELISA Kit #7238

Specificity/Sensitivity: CST's PathScan® Acetylated p53 Sandwich ELISA Kit detects endogenous levels of Acetylated p53. Using this Sandwich ELISA Kit #7236, acetylated lysines on p53 are detected when treated with TSA in COS cells. However, the levels of p53 remain unchanged, as shown by Western analysis (Figure 1). NIH/3T3 and 293 cells treated with TSA show similar results (data not shown).

Products Included	Volume	Solution Color
p53 Rabbit Antibody Coated Microwells*	96 tests	
Acetylated-Lysine 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).

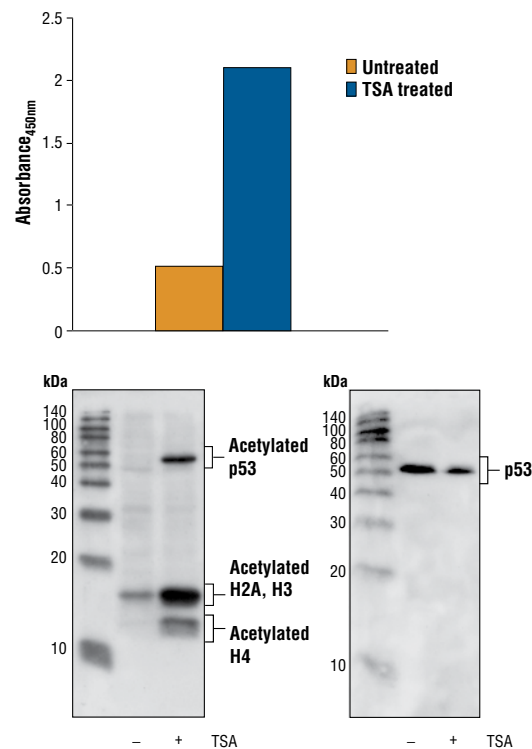


Figure 1: Treatment of COS cells with TSA causes accumulation of acetylation on p53, detected by Sandwich ELISA Kit #7236, but does not affect the level of p53 protein, detected by Western analysis. The absorbance at 450 nm is shown in the top figure, while the corresponding Western blot using the Acetylated Lysine mouse mAb (Ac-K-103) #9681 (left panel) or p53 Antibody #2524 (right 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 between p53 and its negative regulator, oncoprotein MDM2 (4). MDM2 inhibits p53 accumulation 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. Phosphorylation impairs the ability of MDM2 to bind p53, promoting both the accumulation and 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 regulates the ability of p53 to induce apoptosis (16). Acetylation of p53 is mediated by p300 and CBP acetyltransferases. Inhibition of deacetylation suppressing MDM2 from recruiting HDAC1 complex by p19 (ARF) stabilizes p53. Acetylation appears to play a positive role in the accumulation of p53 protein in stress response (17). Following DNA damage, Human p53 becomes acetylated at Lys382 (Lys379 in mouse) *in vivo* to enhance p53-DNA binding (18). Deacetylation of p53 occurs through interaction with the SIRT1 protein, a deacetylase that may be involved in cellular aging and the DNA damage response (19).

Background References:

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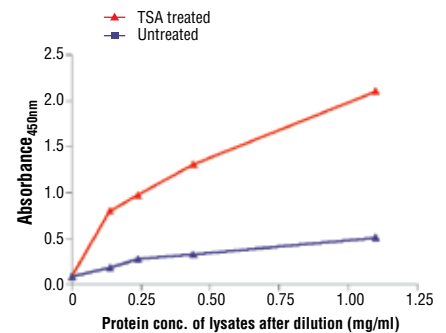


Figure 2: The relationship between protein concentration of lysates from untreated and TSA treated COS cells and the absorbance at 450 nm is shown. COS cells (80% confluence) were treated with TSA (0.4 μ M overnight).

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.