

PathScan® Phospho-p53 (Ser15) Sandwich ELISA Antibody Pair

✓ 1 Kit
(4 X 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.
This product is not intended for use as a therapeutic or in diagnostic procedures.

Entrez-Gene ID #7157
Swiss-Prot Acc. #P04637

Species Cross-Reactivity: H

Description: CST's PathScan® Phospho-p53 (Ser15) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-p53 (Ser15) Sandwich ELISA Kit #7365. Capture and Detection antibodies (100X stocks) and HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The p53 Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysates are added followed by a Phospho-p53 (Ser15) Detection Antibody and anti-Mouse IgG, HRP conjugated antibody. HRP substrate, TMB, is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of phospho-p53 (Ser15) protein.

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

Reagents not supplied:

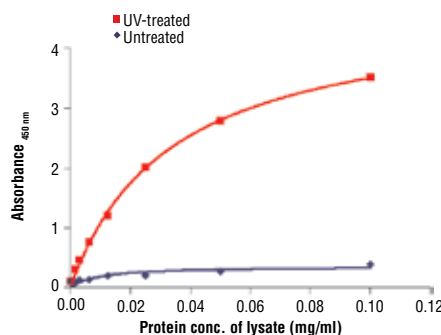
- Phosphate Buffered Saline (PBS-20X) #9808
- Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809
- Cell Lysis Buffer (10X) #9803
- TMB Substrate #7004
- STOP Solution #7002
- Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA
- 96 Well Microplates**
- Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Notes: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

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 a reduced interaction between p53 and its negative regulator, the oncoprotein MDM2 (4). MDM2 inhibits p53 accumulation by targeting it for ubiquitination and proteasomal degradation (6,7). p53 can be phosphorylated by ATM, ATR and DNA-PK at Ser15 and Ser37. Phosphorylation impairs

Products Included	Volume	Cap Color	Storage
p53 Rabbit Capture Antibody (100X)	0.4 ml	Pink	4°C
p53 (Ser15) Mouse Detection Antibody (100X)	0.4 ml	Blue	4°C
Anti-Mouse IgG, HRP-Linked Antibody (1000X)	0.04 ml	Yellow	-20°C



The relationship between protein concentration of lysates from untreated and UV-treated HT-29 cells and the absorbance at 450 nm using PathScan® Phospho-p53 (Ser15) Sandwich ELISA Antibody Pair #7846 is shown. HT-29 cells (80% confluent) were UV-treated, incubated at 37°C for 2 hours and then lysed.

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 increased 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 CK1δ and CK1ε 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).

Storage: Capture and Detection Antibodies are stored at 4°C. Anti-Mouse IgG, HRP-Linked Antibody is stored at -20°C.

Companion Products:

- PathScan® Acetylated p53 Sandwich ELISA Kit #7236
- PathScan® Phospho-p53 (Ser15) Sandwich ELISA Kit #7365
- PathScan® Total p53 Sandwich ELISA Kit #7370
- BSA #9998
- Anti-mouse IgG, HRP-linked Antibody #7076

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

Background References:

- (1) Levine, A.J. (1997) *Cell* 88, 323–331.
- (2) Meek, D.W. (1994) *Semin. Cancer Biol.* 5, 203–210.
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- (12) Lu, H. et al. (1997) *Mol. Cell. Biol.* 17, 5923–5934.
- (13) Lohrum, M. and Scheidtmann, K.H. (1996) *Oncogene* 13, 2527–2539.
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- (16) Oda, K. et al. (2000) *Cell* 102, 849–862.
- (17) Ito, A. et al. (2001) *EMBO J.* 20, 1331–1340.
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PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)
3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #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.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

- Rinse microplate with dH₂O. Add 200 μl of dH₂O and discard liquid. Blot on paper towel to make sure wells are dry.
- Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 μl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 μl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
- After overnight coating, gently uncover plate and wash wells:**
 - Discard plate contents into a receptacle.
 - Wash 4 times with 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.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

- Aspirate media, treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenylmethylsulfonyl fluoride (PMSF) to each plate (10 cm diameter plate) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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.

D Test Procedure

- Lysates can be used undiluted or diluted in Blocking Buffer. 100 μl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
- Wash plate as in Coating Procedure, Step 3.
- Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 μl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover plate and incubate at 37°C for 1 hour.
- Plate is washed as in Coating Procedure, Step 3.
- Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 μl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover and incubate at 37°C for 30 minutes.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.