Store at 4°C

#7105

PathScan® Apoptosis Multi-Target Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

rev. 02/24/16

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

Species Cross-Reactivity: H, Mk

Introduction: CST's PathScan® Apoptosis Multi-Target Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that combines the reagents necessary to detect endogenous levels of p53 protein, phospho-p53 protein (Ser15), Bad, phospho-Bad (Ser112), Cleaved Caspase-3 (Asp175) and Cleaved PARP (Asp214). These molecules represent key signaling proteins in pathways controlling survival and apoptosis. Sixteen assays are provided for each target protein. Specific assay formulations for the indicated target proteins can be found in the datasheets associated with the individual sandwich ELISA kits. Briefly, a capture antibody has been coated onto the microwells. After incubation with cell lysates, the target protein is captured by the coated antibody. Following extensive washing, a detection antibody is added to detect the captured target protein. An HRP-linked secondary antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for this developed color is proportional to the quantity of bound target protein.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: Specificity/Sensitivity: CST's PathScan® Apoptosis Multi-Target Sandwich ELISA Kit #7105 detects endogenous levels of six proteins: total p53, phospho-p53 (Ser15), total Bad, phospho-Bad (Ser112), cleaved caspase-3 (Asp175) and cleaved PARP (Asp214). Activation of these proteins can be observed over time in response to toxic chemical compounds. As shown in Figures 1 and 2, both doxorubicin and staurosporine can induce apoptosis in HeLa cells, evidenced by increased levels of cleaved PARP and caspase-3. However, treatment with doxorubicin, which damages cellular DNA, induces p53 phosphorylation at Ser15 and stabilizes p53, while treatment with staurosporine, a kinase inhibitor, has no effect on p53 phosphorylation. While total Bad and phospho-Bad (Ser112) levels are relatively consistent after doxorubicin treatment, a gradual decline of both targets was observed after staurosporine treatment. COS cells are resistant to apoptosis due to high constitutive levels of p53. Therefore, the same dose of doxorubicin applied

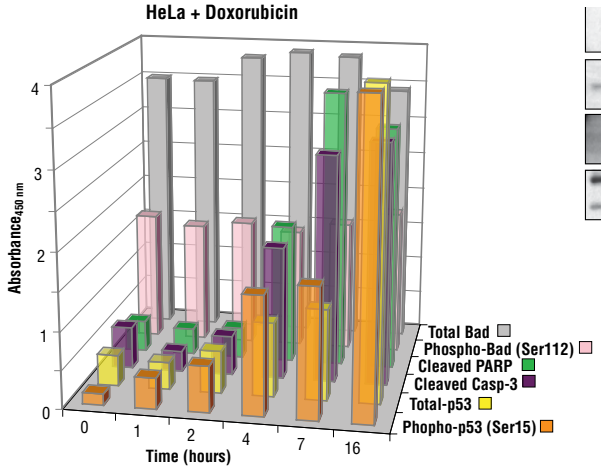
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Products Included	Quantity	Solution Color	Cap Color
p53 Rabbit mAb Coated MicroWells	16 tests		
Phospho-p53 (Ser15) Detection Antibody	1.8 ml	Green	**Orange**
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	**Orange**
p53 Rabbit mAb Coated MicroWells	16 tests		
p53 Detection Antibody	1.8 ml	Green	**Yellow**
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	**Yellow**
Cleaved-Caspase-3 (Asp175) Rabbit mAb Coated Microwells	16 tests		
Biotinylated Caspase-3 Rabbit Detection Antibody	1.8 ml	Green	**Purple**
HRP-linked Streptavidin	1.8 ml	Red	**Purple**
PARP (Asp214) Antibody Coated Microwells	16 tests		
PARP Biotinylated Detection Antibody	1.8 ml	Green	**Green**
HRP- Linked Streptavidin	1.8 ml	Red	**Green**
Bad Antibody-Coated Microwells	16 tests		
Phospho-Bad (Ser112) Detection Antibody	1.8 ml	Green	**Pink**
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	**Pink**
Bad Antibody-Coated Microwells	16 tests		
Bad Detection Antibody	1.8 ml	Green	**Grey**
Anti-Mouse IgG HRP-Linked Antibody	1.8 ml	Red	**Grey**
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	
Cell Lysis Buffer (10X)	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).

U.S. Patent No. 5,675,063

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% homology.



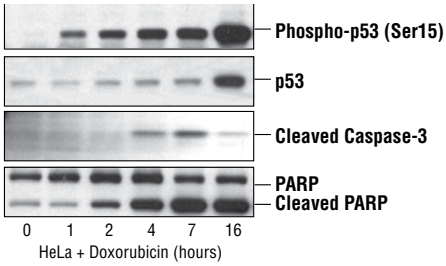
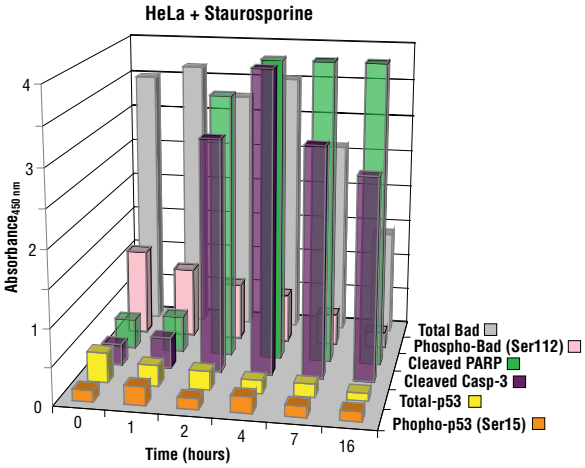


Figure 1. Treatment of HeLa cells with doxorubicin induces phosphorylation of p53 at Ser15, as well as cleavage of PARP and caspase-3 as detected by PathScan® Apoptosis Multi-Target Sandwich ELISA Kit #7105 and Western analysis. HeLa cells (80-90% confluent) were starved overnight and stimulated with doxorubicin (5 µM at 37°C for indicated times). Lysates were assayed at a protein concentration of 1 mg/ml. The absorbance readings at 450 nm are shown as a 3-dimensional representation in the left panel, while the corresponding Western blots are shown in the right panel. Antibodies used for Western analysis include Phospho-p53 (Ser15) Antibody #9284, p53 Antibody #9282, Cleaved Caspase-3 (Asp175) Antibody #9661 and PARP Antibody #9542. Total Bad and phospho-Bad (Ser112) proteins were not detected by Western due to low endogenous levels in HeLa cells.



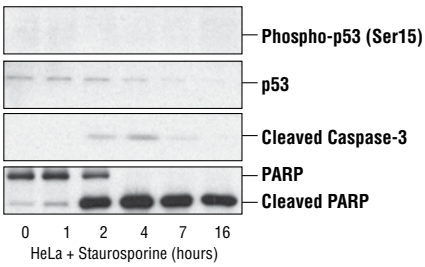
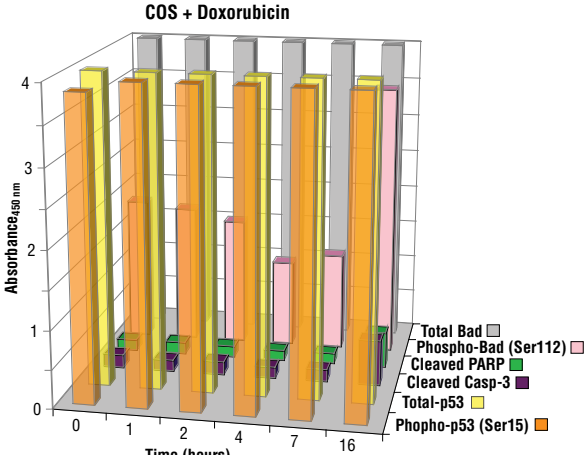


Figure 2. Treatment of HeLa cells with staurosporine induces cleavage of PARP and caspase-3 but not phosphorylation of p53 at Ser15 as detected by PathScan® Apoptosis Multi-Target Sandwich ELISA Kit #7105 and Western analysis. HeLa cells (80-90% confluent) were starved overnight and stimulated with staurosporine (2 µM at 37°C for indicated times). Lysates were assayed at a protein concentration of 1 mg/ml. The absorbance readings at 450 nm are shown as a 3-dimensional representation in the left panel, while the corresponding Western blots are shown in the right panel. The antibodies used for the Western analyses include Phospho-p53 (Ser15) Antibody #9284, p53 Antibody #9282, Cleaved Caspase-3 (Asp175) Antibody #9661 and PARP Antibody #9542. Total Bad and Phospho-Bad (Ser112) proteins were not detected by Western due to low endogenous levels in HeLa cells.



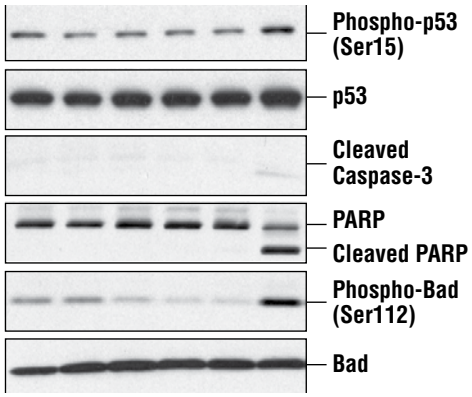


Figure 3. Treatment of COS cells with doxorubicin induces low levels apoptosis as detected by PathScan® Apoptosis Multi-Target Sandwich ELISA Kit #7105 and Western analysis. COS cells (80-90% confluent) were starved overnight and stimulated with doxorubicin (5 µM for at 37°C for indicated times). Lysates were assayed at a protein concentration of 1 mg/ml. The absorbance readings at 450 nm are shown as a 3-dimensional representation in the left panel, while the corresponding Western blots are shown in the right panel. The antibodies used for the Western analyses include Phospho-p53 (Ser15) Antibody #9284, p53 Antibody #9282, Cleaved Caspase-3 (Asp175) Antibody #9661, PARP Antibody #9542, Phospho-Bad (Ser112) (7E11) Mouse mAb #9296 and Bad Antibody #9292.

Cell Signaling TECHNOLOGY®

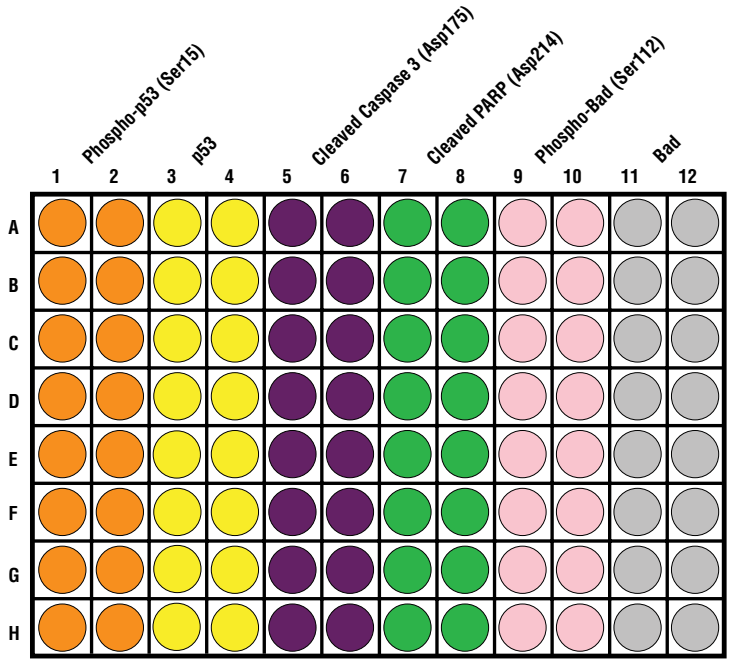


Figure 4. Schematic representation of a 96-well plate depicting the color-code of the reagents used to detect endogenous levels of Phospho-p53 (Ser15) (orange; 1 & 2), p53 (yellow; 3 & 4), Cleaved Caspase 3 (Asp175) (purple; 5 & 6), Cleaved PARP (Asp214) (green; 7 & 8), Phospho-Bad (Ser112) (pink; 9 & 10) and Bad protein (grey; 11 & 12).

Background: Apoptosis is a regulated physiological process leading to cell death. Caspases, a family of cysteine acid proteases, are central regulators of apoptosis. Initiator caspases (including 8, 9, 10 and 12) are closely coupled to proapoptotic signals. Once activated, these caspases cleave and activate downstream effector caspases (including 3, 6 and 7), which in turn cleave cytoskeletal and nuclear proteins like PARP, α-fodrin, DFF and lamin A, and induce apoptosis. Cytochrome c released from mitochondria is coupled to the activation of caspase-9, a key initiator caspase (1). Proapoptotic stimuli include the FasL, TNF-α, DNA damage and ER stress. Fas and TNFR activate caspases 8 and 10 (2), DNA damage leads to the activation of caspase-9 and ER stress leads to the calcium-mediated activation of caspase-12 (3). The inhibitor of apoptosis protein (IAP) family includes XIAP and survivin and functions by binding and inhibiting several caspases (4,5). Smac/Diablo, a mitochondrial protein, is released into the cytosol upon mitochondrial stress and competes with caspases for binding of IAPs. The interaction of Smac/Diablo with IAPs relieves the inhibitory effects of the IAPs on caspases (6).

Background References:

(1) Baker, S.J. and Reddy, E.P. (1998) *Oncogene* 17, 3261-3270.

(2) Budihardjo, I. et al. (1999) *Annu. Rev. Cell Dev. Biol.* 15, 269-290.

(3) Nakagawa, T. et al. (2000) *Nature* 403, 98-103.

(4) Deveraux, Q. L. et al. (1998) *EMBO J.* 17, 2215-2223.

(5) Li, F. et al. (1998) *Nature* 396, 580-584.

(6) Du, C. et al. (2000) *Cell* 102, 33-42.

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-colored solution) to each well. **Be sure to match the cap color of the Detection Antibody with the corresponding color code of the 8-well strip.** 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-colored solution) to each well. **Be sure to match the cap color of the Detection Antibody with the corresponding color code of the 8-well strip.** 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.