Revision 1

#7105 Store at 4°C

PathScan® Apoptosis Multi-Target Sandwich ELISA Kit

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Species Cross Reactivity	UniProt ID:	Entrez-Gene Id:
H Mk	#P04637, #Q92934, #P42574, #P09874	#7157, #572, #836, #142

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Product Includes	Product #	Quantity	Color	Storage Temp
Apoptosis Multi-Target	43136	96 tests		4°C
TMB Substrate	7004	11 ml	Colorless	4°C
STOP Solution	7002	11 ml	Colorless	4°C
Sealing Tape	54503	2 ea		4°C
ELISA Wash Buffer (20X)	9801	25 ml	Colorless	4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml	Yellowish	-20°C

*The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

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.
*See companion products.
**Antibodies in kit are custom formulations specific to kit.

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 to HeLa cells only induces low amounts of apoptosis in these cells as evidenced by cleaved caspase-3 and cleaved PARP protein levels (Figure 3). The relationship between the protein concentration of the lysate and the absorbance at 450 nm can be found in the datasheets associated with the individual PathScan® Sandwich ELISA Kits*.
*See companion products.
This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

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 FasL, TNF-α, DNA damage and ER stress. Fas and TNFR activate caspase-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 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.

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **Mi:** mink **C:** chicken **Dm:** D. melanogaster **X:** Xenopus **Z:** zebrafish **B:** bovine **Dg:** dog **Pg:** pig **Sc:** S. cerevisiae **Ce:** C. elegans **Hr:** horse **GP:** Guinea Pig **Rab:** rabbit **All:** all species expected

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#7105

PathScan® Apoptosis Multi-Target Sandwich ELISA Kit

ELISA Colorimetric

NOTE: Refer to product-specific datasheets or product webpage for assay incubation temperature.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS)**: (#9808) To prepare 1 L PBS: add 50 ml 10X PBS to 950 ml dH_2O, mix.
2. Bring all microwell strips to room temperature before use.
3. Prepare 1X Wash Buffer by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in dH_2O.
4. **1X Cell Lysis Buffer**: 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH_2O, mix. Buffer can be stored at 4°C for short-term use (1–2 weeks).

   Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

   NOTE: Refer to product-specific datasheet or webpage for lysis buffer recommendation.

5. **TMB Substrate**: (#7004).
6. **STOP Solution**: (#7002).

B. Preparing Cell Lysates

For adherent cells

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X cell lysis buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (x14,000 rpm) 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.

For suspension cells

1. Remove media by low speed centrifugation (~1,200 rpm) when the culture reaches 0.5–1.0 x 10^6 viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1,200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X cell lysis buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (x14,000 rpm) 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 in the storage bag and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with sample diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets or product webpage for each kit provide information regarding an appropriate dilution factor for lysates and kit assay results.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
4. **Gently remove the tape and wash wells**:
   1. Discard plate contents into a receptacle.
   2. Wash 4 times with 1X wash buffer, 200 µl each time per well.
   3. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
   4. 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 at 37°C for 1 hr.
6. Repeat wash procedure (Section C, 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 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min 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**
    1. **Visual Determination**: Read within 30 min after adding STOP solution.
    2. **Spectrophotometric Determination**: Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

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