

PathScan® Total Survivin Sandwich ELISA Kit

✓ 1 Kit
(96 assays)

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This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Entrez-Gene ID # 332
Swiss-Prot Acc. # O15392

Species Cross-Reactivity: H, Mk

Description: CST's PathScan® Total Survivin Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total survivin protein. A Survivin Mouse mAb* has been coated onto the microwells. After incubation with cell lysates, both phospho and nonphospho survivin proteins are captured by the coated antibody. Following extensive washing, Survivin Rabbit Detection Antibody* is added to detect the captured survivin protein. Anti-rabbit IgG, HRP-linked Antibody #7074* 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 total survivin protein.

* Antibodies in kit are custom formulations specific to kit.

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

Specificity/Sensitivity: CST's PathScan® Total Survivin Sandwich ELISA Kit #7169 detects endogenous levels of total survivin protein. As shown in Figure 1, survivin protein from human (HeLa, H526 and MCF-7) and monkey (COS), is detected by this kit. In Figure 3, Western analysis of protein captured in microwells coated with the survivin capture antibody shows a major band corresponding to the survivin protein (data not shown).

Background: Survivin is a 16 kDa anti-apoptotic protein highly expressed during fetal development and cancer cell malignancy (1). Survivin binds and inhibits caspase-3, controlling the checkpoint in the G2/M-phase of the cell cycle through inhibiting apoptosis and promoting cell division (2,3). This regulatory process requires the phosphorylation of survivin at Thr34 by p34 cdc2 kinase (4). Gene targeting using a Thr34 phosphorylation-defective survivin mutant, as well as antisense survivin, has been shown to inhibit tumor growth (5,6).

Background References:

- Reed, J.C. and Reed, S.I. (1999) *Nature Cell Biol.* 1, 199–200.
- Li, F. et al. (1998) *Nature* 396, 580–584.
- Li, F. et al. (1999) *Nature Cell Biol.* 1, 461–466.
- O'Connor, D.S. et al. (2000) *Proc. Natl. Acad. Sci. USA* 97, 13103–13107.
- Olie, R.A. et al. (2000) *Cancer Res.* 60, 2805–2809.
- Grossman, D. et al. (2001) *Proc. Natl. Acad. Sci. USA* 98, 635–640.

Products Included	Volume	Solution Color
Survivin Mouse mAb Coated Microwells*	96 tests	
Survivin Rabbit Detection Antibody	11 ml	green
Anti-rabbit 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).

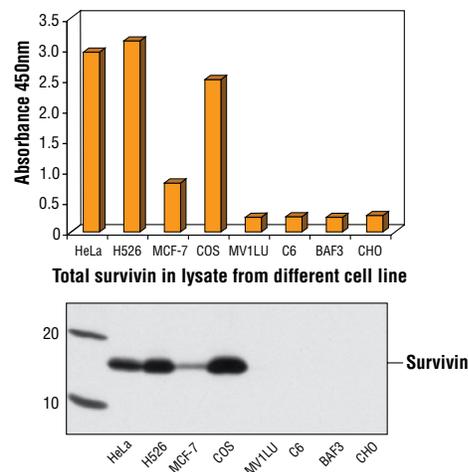


Figure 1: Survivin protein from human cells (HeLa, H526 and MCF-7) and monkey cells (COS) can be detected by PathScan® Total Survivin Sandwich ELISA Kit #7169. However, this kit cannot detect the survivin protein from other species, such as mink (MV1LU), rat (C6), mouse (BAF3) and hamster (CHO) cell lines. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blot using Survivin (6E4) Mouse mAb #2802, is shown in the bottom figure.

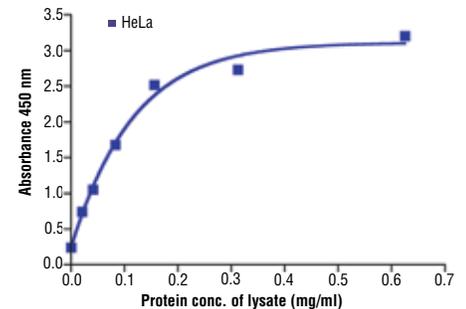


Figure 2: The relationship between protein concentration of lysates from HeLa cells and the absorbance at 450 nm is shown. HeLa cells (80% confluence) were harvested and then lysed.

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 , 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

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.