

# PathScan® Total HSP27 Sandwich ELISA Kit

✓ 1 Kit  
(96 assays)

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rev. 03/03/10

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## Species Cross-Reactivity: H, Mk

**Introduction:** CST's PathScan® Total HSP27 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of total HSP27 protein. An HSP27 Antibody has been coated onto the microwells. After incubation with cell lysates, both nonphospho- and phospho-HSP27 are captured by the coated antibody. Following extensive washing, an HSP27 Mouse mAb is added to detect the captured HSP27 protein. HRP-linked anti-mouse antibody #7076\* is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of optical density for this developed color is proportional to the quantity of total HSP27 protein.

\* Antibodies in kit are custom formulations specific to kit.

Please visit [www.cellsignal.com](http://www.cellsignal.com) for a complete listing of recommended companion products.

**Specificity/Sensitivity:** CST's PathScan® Total HSP27 Sandwich ELISA Kit detects endogenous levels of total HSP27 protein. Using PathScan® Phospho-HSP27 (Ser78) Sandwich ELISA Kit #7290, a significant induction of phospho-HSP27 (Ser78) in HeLa cells treated with UV light can be detected. However, the level of total HSP27 (phospho and non-phospho), detected by this Sandwich ELISA Kit #7295, remains unchanged (Figure 1). COS cells treated with UV light show similar results (data not shown).

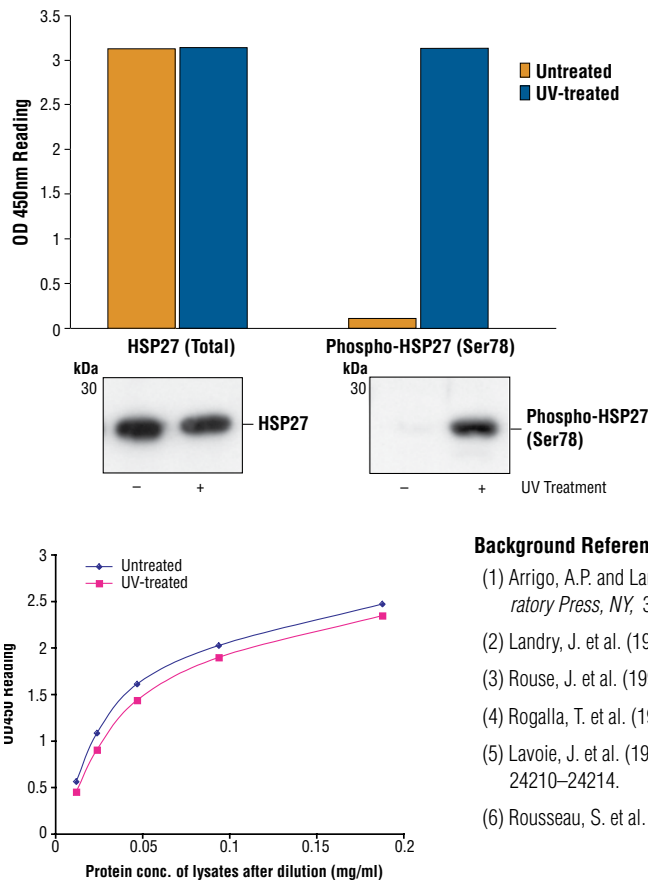
**Background:** Heat shock protein (HSP) 27 is one of the small HSPs that are constitutively expressed at different levels in various cell types and tissues. Like other small heat shock proteins, HSP27 is regulated at both the transcriptional and posttranslational levels (1). In response to stress, the expression level of HSP27 increases several-fold to confer cellular resistance to the adverse environmental change. HSP27 is phosphorylated at Ser15, Ser78 and Ser82 by MAPKAP kinase 2 as a result of the activation of the p38 MAP kinase pathway (2,3). Phosphorylation of HSP27 causes a change in its tertiary structure, which shifts from large homotypic multimers to dimers and monomers (4). It has been shown that phosphorylation and increased concentration of HSP27 modulates actin polymerization and reorganization (5,6).

Figure 2: Relationship between protein concentration of lysates from untreated and UV-treated HeLa cells and kit assay optical density readings. HeLa cells (70-85% confluence) were treated with UV and lysed after incubation at 37°C for 30 minutes.

Products Included	Volume	Solution Color
HSP27 Antibody Coated Microwells*	96 tests	
HSP27 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 HeLa cells with UV light stimulates phosphorylation of HSP27 at Ser78, detected by PathScan® Phospho-HSP27 (Ser78) Sandwich ELISA kit, #7290. OD<sub>450</sub> readings (upper) and the corresponding Western blot using Phospho-HSP27 (Ser78) Ab #2405 (lower right) or HSP27 (G31) Mouse mAb #2402 (lower left), are shown.

## Background References:

- (1) Arrigo, A.P. and Landry, J. (1994) *Cold Spring Harbor Laboratory Press, NY*, 335-373.
- (2) Landry, J. et al. (1992) *J. Biol. Chem.* 267, 794-803.
- (3) Rouse, J. et al. (1994) *Cell* 78, 1027-1037.
- (4) Rogalla, T. et al. (1999) *J. Biol. Chem.* 274, 18947-18956.
- (5) Lavoie, J. et al. (1993) *J. Biol. Chem.* 268, 24210-24214.
- (6) Rousseau, S. et al. (1997) *Oncogene* 15, 2169-2177.

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E—ELISA

**Species Cross-Reactivity Key:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus Z—zebra fish B—bovine Dg—Dog All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

## 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<sub>3</sub>VO<sub>4</sub>, 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.