

PathScan® Total β -Actin Sandwich ELISA Kit

✓ 1 Kit
(96 assays)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

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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 #60
Swiss-Prot Acc. #P60709

Species Cross-Reactivity: H, M, R, Mk, Hm

Description: The PathScan® Total β -Actin Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of β -actin. A β -actin rabbit antibody has been coated onto the microwells. After incubation with cell lysates, β -actin is captured by the coated antibody. Following extensive washing, a pan-actin mouse detection antibody is added to detect the captured β -actin. An anti-mouse IgG, HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate (TMB) is added to develop color. The magnitude of the absorbance for this developed color is proportional to the quantity of β -actin.

Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Total β -Actin Sandwich ELISA Kit detects endogenous levels of β -actin. As shown in Figure 1, β -actin is readily detected in HeLa cells using the PathScan® Total β -Actin Sandwich ELISA Kit. Total levels of β -actin remain unchanged after IFN- α treatment as shown by western analysis. The PathScan® Total β -Actin Sandwich ELISA Kit does not cross-react with α -smooth muscle actin, α -sarcomeric muscle actin or γ -actin. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

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

Products Included	Volume	Solution Color
β -Actin Rabbit Antibody Coated Microwells*	96 tests	
Pan-Actin Mouse 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).

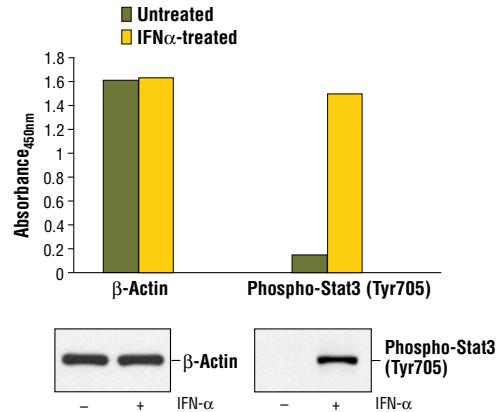


Figure 1. Treatment of HeLa cells with IFN- α stimulates phosphorylation of Stat3 at Tyr705 as detected by PathScan® Phospho-Stat3 (Tyr705) Sandwich ELISA Kit #7300, but does not affect the level of β -actin as detected by PathScan® Total β -Actin Sandwich ELISA Kit #7880. HeLa cells were treated with 100 ng/ml IFN- α for ten minutes at 37°C before lysis. Absorbance at 450 nm is shown in the top figure, while the corresponding western blots using Phospho-Stat3 (Tyr705) (3E2) Mouse mAb #9138 (right panel) or Total β -Actin (13E5) Rabbit mAb #4970 (left panel) are shown in the bottom figure.

Background: Actin, an ubiquitous protein in eukaryotes, is the major component of the cytoskeleton. At least six isoforms are known in mammals. Nonmuscle β - and γ -actin, also known as cytoplasmic actin, are predominantly expressed in nonmuscle cells, controlling cell structure and motility (1). α -cardiac and α -skeletal actin are expressed in striated cardiac and skeletal muscles, respectively; two smooth muscle actins, α - and γ -actin, are found primarily in vascular smooth muscle and enteric smooth muscle, respectively. These actin isoforms regulate contractile potentials for muscle cells (1). Actin exists mainly as a fibrous polymer, F-actin. In response to cytoskeletal reorganizing signals during processes such as cytokinesis, endocytosis, or stress, cofilin promotes fragmentation and depolymerization of F-actin, resulting in an increase in the monomeric globular form, G-actin (2). The Arp2/3 complex stabilizes F-actin fragments and promotes formation of new actin filaments (2). It has been reported that actin is hyperphosphorylated in primary breast tumors (3). Cleavage of actin under apoptotic conditions has been observed *in vitro* and in cardiac and skeletal muscle (4-6). Actin cleavage by caspase-3 may accelerate ubiquitin/proteasome dependent muscle proteolysis (6).

Background References:

- (1) Herman, I.M. (1993) *Curr. Opin. Cell Biol.* 5, 48–55.
- (2) Condeelis, J. (2001) *Trends Cell Biol.* 11, 288–293.
- (3) Lim, Y.P. et al. (2004) *Clin. Cancer Res.* 10, 3980–3987.
- (4) Kayalar, C. et al. (1996) *Proc. Natl. Acad. Sci. USA.* 93, 2234–2238.
- (5) Communal, C. et al. (2002) *Proc. Natl. Acad. Sci. USA.* 99, 6252–6256.
- (6) Du, J. et al. (2004) *J. Clin. Invest.* 113, 115–123.

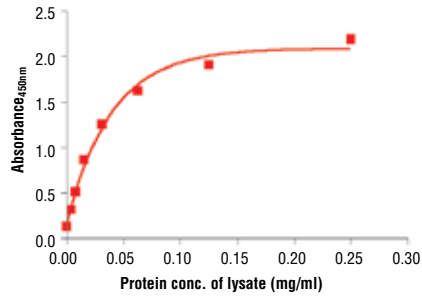


Figure 2. The relationship between the protein concentration of the lysate from HeLa cells and the absorbance at 450 nm is shown.

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.