

# PathScan® Phospho-IRS-1 (Ser302) 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. 03/21/14

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

## Species Cross-Reactivity: H

**Introduction:** The PathScan® Phospho-IRS-1 (Ser302) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of IRS-1 when phosphorylated at Ser302. An IRS-1 Mouse Antibody\* has been coated onto the microwells. After incubation with cell lysates, IRS-1 (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Phospho-IRS-1 (Ser302) Rabbit Detection Antibody\* is added to detect phosphorylation of Ser302 on the captured IRS-1 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 the absorbance for this developed color is proportional to the quantity of IRS-1 phosphorylated at Ser302.

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

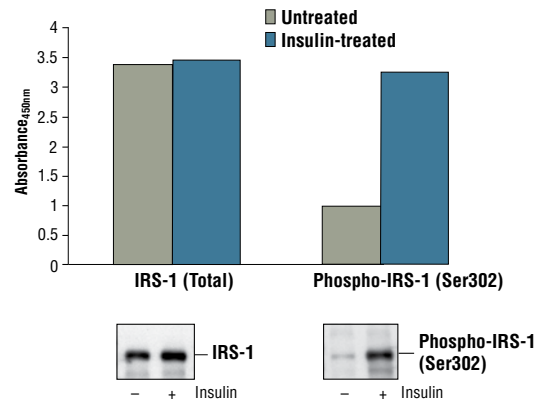
## Companion Products:

PathScan® Total IRS-1 Sandwich ELISA Kit #7328  
PathScan® Phospho-IRS-1 (Ser612) Sandwich ELISA Kit #7332  
PathScan® Phospho-IRS-1 (panTyr) Sandwich ELISA Kit #7133  
PathScan® Phospho-IRS-1 (Ser307) Sandwich ELISA Kit #7287  
PathScan® Phospho-IRS-1 (Ser302) Sandwich ELISA Antibody Pair #7284  
IRS-1 Antibody #2382  
IRS-1 (59G8) Rabbit mAb #2390  
IRS-1 (L3D12) Mouse mAb #3194  
Phospho-IRS-1 (Ser302) (34C7) Rabbit mAb #2491  
Phospho-IRS-1 (Ser302) Antibody #2384  
Anti-rabbit IgG, HRP-linked Antibody #7074  
TMB Substrate #7004  
STOP Solution #7002  
Phosphate Buffered Saline (PBS-20X) #9808  
Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

Products Included	Volume	Solution Color
IRS-1 Mouse Ab Coated Microwells*	96 tests	
Phospho-IRS-1 (Ser302) Rabbit Detection Ab	11 ml	green
Anti-Rabbit IgG HRP-Linked Ab	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 hSkMC cells with insulin stimulates phosphorylation of IRS-1 at Ser302, detected by the PathScan® Phospho-IRS-1 (Ser302) Sandwich ELISA Kit #7283, but does not affect the level of total IRS-1 detected by PathScan® Total IRS-1 Sandwich ELISA Kit #7328. hSkMC cells (80-90% confluent) were starved overnight and treated with 100 nM insulin for 15 minutes at 37°C. The absorbance readings at 450 nm are shown in the top figure, while the corresponding Western blots using IRS-1 (L3D12) Mouse mAb #3194 (left panel) or Phospho-IRS-1 (Ser302) (34C7) Rabbit mAb #2491 (right panel) are shown in the bottom figure.

**Specificity/Sensitivity:** CST's PathScan® Phospho-IRS-1 (Ser302) Sandwich ELISA Kit #7283 detects endogenous levels of IRS-1 when phosphorylated at Ser302. As shown in Figure 1, a significant induction of IRS-1 phosphorylation at Ser302 can be detected in hSkMC cells following treatment with insulin using the Phospho-IRS-1 (Ser302) Sandwich ELISA Kit #7283. The level of total IRS-1 (phospho and nonphospho) remains unchanged as shown by Western analysis and by PathScan® Total IRS-1 Sandwich ELISA Kit #7328 (Figure 1).

U.S. Patent No. 5,675,063  
Tween®20 is a registered trademark of ICI Americas, Inc.  
F—Flow cytometry E—ELISA D—DELFIATM

**Applications Key:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence  
**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 All—all species expected  
Species enclosed in parentheses are predicted to react based on 100% sequence homology.



**Background:** Insulin receptor substrate 1 (IRS-1) is one of the major substrates of the insulin receptor kinase (1). IRS-1 contains multiple tyrosine phosphorylation motifs that serve as docking sites for SH2 domain containing proteins that mediate the metabolic and growth promoting functions of insulin (2-4). IRS-1 also contains over 30 potential serine/threonine phosphorylation sites. Ser307 of IRS-1 is phosphorylated by JNK (5) and IKK (6) and Ser789 is phosphorylated by SIK-2, a member of AMPK family (7). The PKC and mTOR pathways mediate phosphorylation of IRS-1 at Ser612 and Ser636/639, respectively (8,9). Phosphorylation of IRS-1 at Ser1101 is mediated by PKC $\theta$  and results in an inhibition of insulin signaling in the cell, which suggests a potential mechanism for insulin resistance in some models of obesity (10).

#### Background References:

- (1) Sun, X.J. et al. (1991) *Nature* 352, 73-77.
- (2) Sun, X.J. et al. (1992) *J. Biol. Chem.* 267, 22662-22672.
- (3) Myers Jr., M.G. et al. (1993) *Endocrinology* 132, 1421-1430.
- (4) Wang, L.M. et al. (1993) *Science* 261, 1591-1594.
- (5) Rui, L. et al. (1997) *J. Clin. Invest.* 107, 181-189.
- (6) Gao, Z. et al. (2002) *J. Biol. Chem.* 277, 48115-48121.
- (7) Horike, N. et al. (2003) *J. Biol. Chem.* 278, 18440-18447.
- (8) Ozes, O.N. et al. (2001) *Proc. Natl. Acad. Sci. USA* 98, 4640-4645.
- (9) De Fea, K. and Ruth, R.A. (1997) *Biochemistry* 36, 12939-12947.
- (10) Li, Y. et al. (2004) *J. Biol. Chem.* 279, 45304-45307.

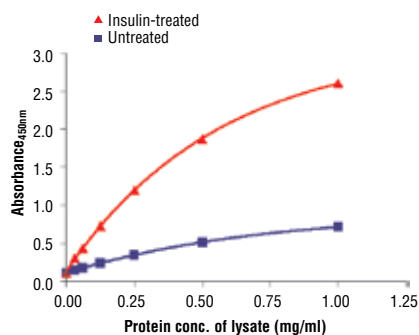


Figure 2. The relationship between the protein concentration of the lysate from untreated and insulin-treated hSkMC cells and the absorbance at 450 nm is shown.

## 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<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<sup>2</sup>) 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. (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.

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 C4.
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 C4.
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.