

# PathScan® Phospho-Insulin Receptor $\beta$ (Tyr1345) 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

New 03/07

This product is for *in vitro* research use only and is not intended for use in humans or animals.

## Species Cross-Reactivity: H, M

**Introduction:** CST's PathScan® Phospho-Insulin Receptor  $\beta$  (Tyr1345) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects transfected phospho-insulin receptor (Tyr1345) protein. Insulin Receptor  $\beta$  (Tyr1345) Rabbit mAb has been coated onto the microwells. After incubation with cell lysates, only phospho-insulin receptor proteins are captured by the coated antibody. Following extensive washing, insulin receptor  $\beta$  mouse mAb is added to detect the captured phospho-insulin receptor (Tyr1345) protein. Anti-mouse IgG, HRP-linked Antibody #7076\* 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 phospho-insulin receptor (Tyr1345) protein.

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

## Companion Products:

PathScan® Phospho-Insulin Receptor (Tyr1146) Sandwich ELISA Kit #7254

PathScan® Phospho-Insulin Receptor (Tyr1150/1151) Sandwich ELISA Kit #7258

Phospho-Insulin Receptor  $\beta$  (Tyr1345) (14A4) Rabbit mAb #3026

Anti-mouse IgG, HRP-linked Antibody #7076

Insulin Receptor  $\beta$  (4B8) Rabbit mAb #3025

Phospho-Insulin Receptor  $\beta$  (Tyr1361) (84B2) Rabbit mAb #3023

Insulin Receptor  $\beta$  (L55B10) Mouse mAb #3020

Phospho-IGF-I Receptor (Tyr1135/1136)/Insulin Receptor (Tyr1150/1151) (19H7) Rabbit mAb #3024

**Specificity/Sensitivity:** CST's PathScan® Phospho-Insulin Receptor  $\beta$  (Tyr1345) Sandwich ELISA Kit #7326 detects phospho-insulin receptor (Tyr1345) protein. As shown in Figure 1, using Phospho-Insulin Receptor  $\beta$  (Tyr1345) Sandwich ELISA Kit #7326, a significant induction of phospho-insulin receptor (Tyr1345) is detected in CHO-IR/IRS-1 cells treated with insulin. The levels of total insulin receptor  $\beta$  (phospho and nonphospho) shown by Western analysis remain unchanged.

Products Included	Volume	Solution Color
Phospho-Insulin Receptor $\beta$ (Tyr1345) Rabbit mAb Coated Microwells*	96 tests	
Insulin Receptor $\beta$ 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).

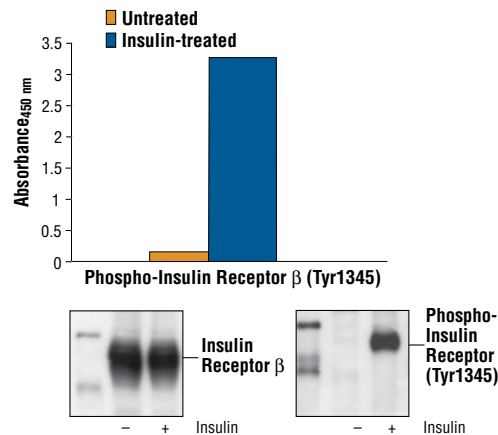


Figure 1: Treatment of CHO-IR/IRS-1 cells with insulin stimulates phosphorylation of insulin receptor at Tyr1345, detected by PathScan® Phospho-Insulin Receptor  $\beta$  (Tyr1345) Sandwich ELISA Kit #7326, but the level of total insulin receptor shown by Western blot analysis remains unchanged. Absorbances at 450 nm are shown in the top figure, while the corresponding Western blots using Phospho-Insulin Receptor  $\beta$  (Tyr1345) Rabbit mAb #3026 (right panel) or Insulin Receptor  $\beta$  Antibody #3025 (left panel), are shown in the bottom figure. CHO-IR/IRS-1 cells stably overexpress human insulin receptor and rat IRS-1.



**Background:** Type I insulin-like growth factor receptor (IGF-IR), a transmembrane receptor tyrosine kinase, is widely expressed in many cell types within fetal and post-natal tissues, and in many cell lines (1-3). Upon binding to its ligands, IGF-I and IGF-II, receptor autophosphorylation occurs. The triple tyrosine cluster within the kinase domain (Tyr1131, Tyr1135 and Tyr1136) is the earliest major site of autophosphorylation (4). Phosphorylation of these three tyrosine residues is necessary for kinase activation (5,6).

Insulin receptors (IRs) share significant similarity with IGF-I receptors in both structure and function, including an equivalent triple tyrosine cluster within the activation loop of the kinase domain (Tyr1146, Tyr1150 and Tyr1151). Tyrosine autophosphorylation of insulin receptor is one of the earliest cellular responses to insulin stimulation (7). Autophosphorylation begins with phosphorylation of Tyr1146 and either Tyr1150 or Tyr1151. Full kinase activation requires the triple tyrosine phosphorylation (8).

#### Background References:

- (1) Adams, T.E. et al. (2000) *Cell. Mol. Life Sci.* 57, 1050–1093.
- (2) Baserga, R. et al. (2000) *Oncogene* 19, 5574–5581.
- (3) Scheidegger, K.J. et al. (2000) *J. Biol. Chem.* 275, 38921–38928.
- (4) Hernandez-Sanchez, C. et al. (1995) *J. Biol. Chem.* 270, 29176–29181.
- (5) Lopaczynski, W. et al. (2000) *Biochem. Biophys. Res. Commun.* 279, 955–960.
- (6) Baserga, R. et al. (1999) *Exp. Cell Res.* 253, 1–6.
- (7) White, M.F. et al. (1985) *J. Biol. Chem.* 260, 9470–9478.
- (8) White, M.F. et al. (1988) *J. Biol. Chem.* 263, 2969–2980.

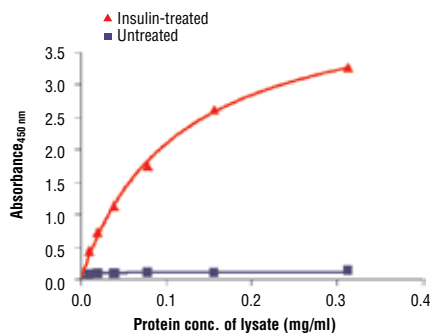


Figure 2: The relationship between protein concentration of lysates from untreated and insulin-treated CHO-IR/IRS-1 cells and the absorbance at 450 nm is shown. After starvation, CHO-IR/IRS-1 cells (85% confluence) were treated with insulin (100 nM) for 2 min at 37°C and then lysed.

## 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<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.