

# PathScan® Phospho-Insulin Receptor $\beta$ (panTyr) Sandwich ELISA Kit



✓ 1 Kit  
(96 assays)

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

## Species Cross-Reactivity: H

**Description:** The PathScan® Phospho-Insulin Receptor  $\beta$  (panTyr) Sandwich ELISA Kit from Cell Signaling Technology is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects transfected levels of tyrosine-phosphorylated Insulin Receptor  $\beta$  protein. An Insulin Receptor  $\beta$  Rabbit mAb has been coated on the microwells. After incubation with cell lysates, Insulin Receptor  $\beta$  protein (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a phospho-tyrosine Mouse mAb is added to detect captured tyrosine-phosphorylated Insulin Receptor  $\beta$  protein. 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 Insulin Receptor  $\beta$  protein phosphorylated on tyrosine.

**Specificity/Sensitivity:** PathScan® Phospho-Insulin Receptor  $\beta$  (panTyr) Sandwich ELISA Kit #7082 detects transfected levels of human Insulin Receptor  $\beta$  protein phosphorylated at Tyrosine, as shown in Figure 1. The kit sensitivity is shown in Figure 2.

**Background:** Insulin receptor (INSR) is a membrane receptor tyrosine kinase. The receptor molecule consists of a disulfide linked heterodimer. The  $\alpha$  subunit is a 135 kDa extracellular fragment, and the  $\beta$  subunit is a 95 kDa fragment containing an extracellular domain, a single transmembrane domain, and an intracellular tyrosine kinase domain (1). Insulin ligand binding to this receptor results in receptor autophosphorylation and tyrosine kinase activation. INSR catalyzes the tyrosine phosphorylation of molecules such as IRS, Gab1, Shc, and Cbl, which further activate the downstream MAPK, PI3K, and TC10 pathways. This eventually leads to increases in glucose uptake and metabolism as well as cell growth (2,3). INSR has peptide substrate specificity similar to other receptor tyrosine kinase members, preferring acidic residues at the -1 to -4 positions and large hydrophobic amino acids at positions +1 and +3 (4).

## Background References:

- Yip, C.C. and Ottensmeyer, P. (2003) *J. Biol. Chem.* 278, 27329-27332.
- Saltiel, A.R. and Pessin, J.E. (2002) *Trends Cell Biol.* 12, 65-71.
- Zick, Y. (2001) *Trends Cell Biol.* 11, 437-441.
- Songyang, Z. and Cantley, L.C. (1995) *TIBS* 20, 470-475.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
Insulin Receptor $\beta$ Rabbit mAb Coated Microwells*	18872	96 tests		4°C
Phospho-Tyrosine Mouse Detection mAb	12982	1 each	Green (Lyophilized)	4°C
Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated)	13304	1 each	Red (Lyophilized)	4°C
Detection Antibody Diluent	13339	11 ml	Green	4°C
HRP Diluent	13515	11 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
Sealing Tape	54503	2 sheets		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
ELISA Sample Diluent	11083	25 ml	Blue	4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

\*12 8-well modules – Each module is designed to break apart for 8 tests.

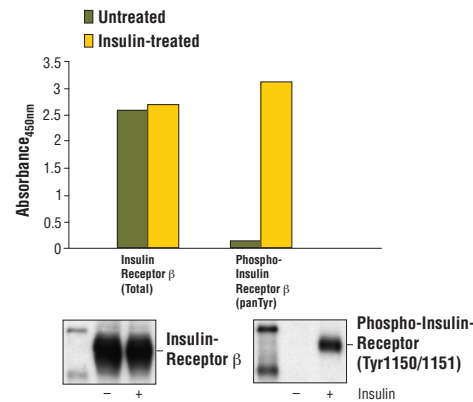


Figure 1: Treatment of CHO-IR/IRS-1 cells with insulin stimulates tyrosine phosphorylation of Insulin Receptor  $\beta$  which is detected by the PathScan® Phospho-Insulin Receptor  $\beta$  (panTyr) Sandwich ELISA Kit #7082 (top, right). Similar levels of Insulin Receptor  $\beta$  protein from both nonphospho or phospho lysates are detected by PathScan® Total Insulin Receptor  $\beta$  Sandwich ELISA Kit #7069 (top, left). Absorbance at 450 nm is shown in the top figure while corresponding western blots using Phospho-IGF-1 Receptor  $\beta$  (Tyr1135/1136)/Insulin Receptor  $\beta$  (Tyr1150/1151) (19H7) Rabbit mAb #3024 (right panel) or Insulin Receptor  $\beta$  Antibody #3025 (left panel) are shown in the bottom figure. CHO-IR/IRS-1 cells stably overexpress the transfected human insulin receptor and rat IRS-1.

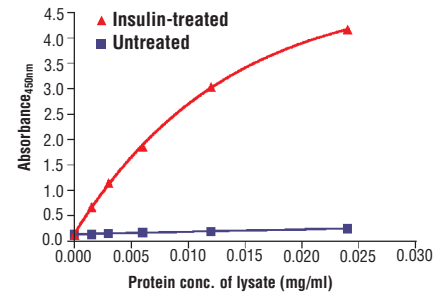


Figure 2: The relationship between protein concentration of lysates from untreated and insulin-treated CHO-IR/IRS-1 cells and the absorbance at 450 nm is shown. After starvation, CHO-IR/IRS-1 cells (85% confluence) were treated with insulin (100 nM, 2 min at 37°C) and then lysed.

# PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

## A Solutions and Reagents

**NOTE:** Prepare solutions with purified water.

1. **Microwell strips:** Bring all to room temperature before use.
2. **Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
3. **HRP-Linked Antibody\*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
4. **Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
5. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
6. **Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
7. **1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
8. **Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803 or 1X Cell Lysis Buffer #7018: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethylsulfonyl fluoride (PMSF) immediately before use.
9. **TMB Substrate** (#7004).
10. **STOP Solution** (#7002).

*\*Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.*

## B Preparing Cell Lysates

### For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) 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.

### For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10<sup>6</sup> viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) 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. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
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 reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min 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 min after adding STOP Solution.
  - b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.