Store at 4°C

#7860C

# PathScan® Phospho-IRS-2 (panTyr) Sandwich ELISA Kit

☑ 1 Kit
(96 assays)

Cell Signaling TECHNOLOGY®

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

**Species Cross-Reactivity: M**

**Introduction:** The PathScan® Phospho-IRS-2 (panTyr) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of IRS-2 when tyrosine phosphorylated. An IRS-2 Rabbit Antibody* has been coated onto the microwells. After incubation with cell lysates, IRS-2 (phospho and nonphospho) is captured by the coated antibody. Following extensive washing, a Phospho-Tyrosine Mouse Detection Antibody* is added to detect tyrosine phosphorylation of the captured IRS-2 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 the absorbance for this developed color is proportional to the quantity of IRS-2 phosphorylated on tyrosine.

* Antibodies in kit are custom formulations specific to kit.

**Specificity/Sensitivity:** CST's PathScan® Phospho-IRS-2 (panTyr) Sandwich ELISA Kit #7860 detects IRS-2 when tyrosine phosphorylated. As shown in Figure 1, a significant induction of IRS-2 tyrosine phosphorylation can be detected in CHO (IR/4PS) cells following treatment with insulin using the Phospho-IRS-2 (panTyr) Sandwich ELISA Kit #7860. The level of total IRS-2 (phospho and nonphospho) remains unchanged as shown by Western analysis. This kit also detects tyrosine phosphorylated IRS-2 in insulin treated 3T3-L1 adipocytes (data not shown).

| Product Includes | Item # | Kit Quantity | Color | Storage Temp |
|---|---|---|---|---|
| IRS-2 Rabbit Antibody Coated Microwells* | 25539 | 96 tests | | 4°C |
| Phospho Tyrosine Mouse Detection mAb | 13951 | 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.

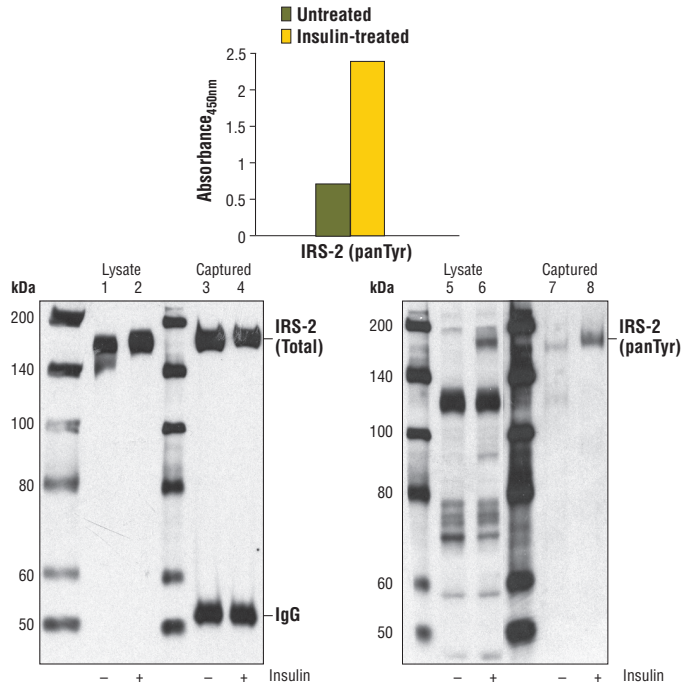


*Figure 1. Treatment of CHO (IR/4PS) cells with insulin stimulates tyrosine phosphorylation of IRS-2, detected by the PathScan® Phospho-IRS-2 (panTyr) Sandwich ELISA Kit #7860, but does not affect the level of total IRS-2 detected by Western analysis. CHO (IR/4PS) cells (80-90% confluent) were starved overnight and treated with 100 nM insulin for 10 minutes at 37°C. The absorbance readings at 450 nm are shown in the top figure. Kit specificity is demonstrated in the bottom figure by Western analysis of the ELISA microwell captured protein. Lysates were prepared from CHO (IR/4PS) cells and incubated in microwells coated with the IRS-2 capture antibody. The wells were washed, and the captured protein was solubilized in SDS gel loading buffer. Western analysis of CHO (IR/4PS) cell starting lysate (lanes 1, 2, 5 & 6) and the captured protein (lanes 3, 4, 7 & 8) was performed using IRS-2 Antibody #4502 (left panel) and Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 (right panel). The major protein detected in the captured material corresponds to IRS-2 (lanes 3, 4 & 8).*



**Applications Key:** **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide
**Species Cross-Reactivity Key:** **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebra fish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% sequence homology.

**Background:** Insulin Receptor Substrate 2 (IRS-2) is one of the major substrates of the insulin receptor kinase (1). In vertebrates, IRS-2 functions as a scaffolding protein to coordinate separate branches of the Insulin/IGF-signaling cascades (2). IRS-2 is essential for normal nutrient homeostasis because it mediates both peripheral insulin action and the effect of IGF-1 on B-cell growth. Mice lacking IRS-2 fail to maintain sufficient compensatory insulin secretion and develop diabetes as young adults (3).

**Background References:**

(1) Sun, X. J. et al. (1991) *Nature* 352, 73–77.

(2) White, M.F. (2002) *Am. J. Phys. Endocrinol. Metab.* 283, E413–E422.

(3) Withers, D.J. et al. (1998) *Nature* 391, 900–904.

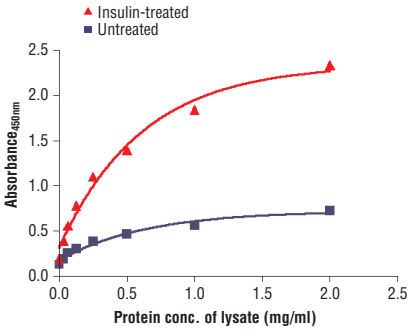


*Figure 2. The relationship between the protein concentration of the lysate from untreated and insulin-treated CHO (IR/4PS) cells and the absorbance at 450 nm is shown.*

# 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: 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 $10^6$ 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.