

#7332C Store at 4°C

PathScan® Phospho-IRS-1 (Ser612) Sandwich ELISA Kit



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1 Kit
 (96 assays)

rev. 02/22/16

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

Species Cross-Reactivity: H, R

Introduction: The PathScan® Phospho-IRS-1 (Ser612) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of IRS-1 when phosphorylated at Ser612. An IRS-1 Rabbit 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 (Ser612) Mouse Detection Antibody* is added to detect phosphorylation of Ser612 on the captured IRS-1 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-1 phosphorylated at Ser612.

* Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Phospho-IRS-1 (Ser612) Sandwich ELISA Kit #7332 detects endogenous levels of Phospho-IRS-1 when phosphorylated at Ser612. As shown in Figure 1, a significant induction of IRS-1 phosphorylation at Ser612 can be detected in hSkMC and CHO (IR/IRS-1) cells following treatment with insulin using the Phospho-IRS-1 (Ser612) Sandwich ELISA Kit #7332. 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).

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, which 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 phosphorylation of Ser612 and Ser636/639 is mediated by the PKC and mTOR pathways, respectively (8,9), and phosphorylation at Ser1101 is mediated by PKCθ (10), resulting in an inhibition of insulin signaling in the cell, suggesting a potential mechanism for insulin resistance in some models of obesity.

Products Included	Volume	Color
IRS-1 Rabbit mAb Coated Microwells*	96 tests	
Phospho-IRS-1 (Ser612) Mouse Detection mAb	1 each	Green (Lyophilized)
Anti-mouse IgG, HRP-linked Antibody	1 each	Red (Lyophilized)
Detection Antibody Diluent	11 ml	Green
HRP Diluent	11 ml	Red
TMB Substrate #7004	11 ml	Colorless
STOP Solution #7002	11 ml	Colorless
Sealing Tape	2 sheets	
20X ELISA Wash Buffer	25 ml	Colorless
ELISA 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).

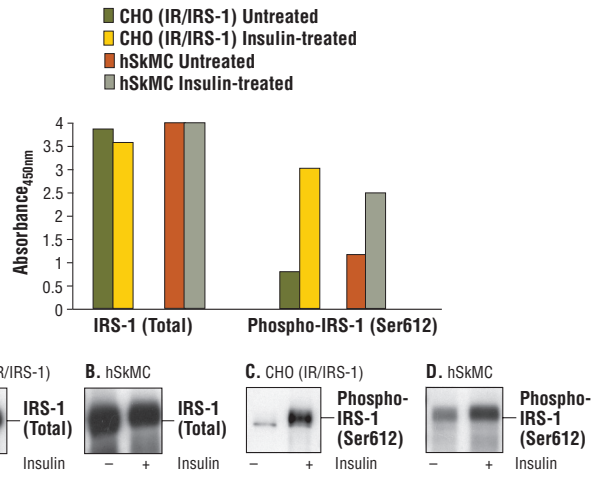


Figure 1. Treatment of hSkMC or CHO (IR/IRS-1) cells with insulin stimulates phosphorylation of IRS-1 at Ser612, detected by the PathScan® Phospho-IRS-1 (Ser612) Sandwich ELISA Kit #7332, but does not affect the level of total IRS-1 detected by PathScan® Total IRS-1 Sandwich ELISA Kit #7328. hSkMC and CHO (IR/IRS-1) cells (80-90% confluent) were starved overnight and treated with 100 nM insulin for 7 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 (panels A & B) or Phospho-IRS-1 (Ser612) (L7B8) Mouse mAb #3193 (panels C & D), are shown in the bottom figure.

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Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
 F—Flow cytometry E—ELISA D—DELFIATM
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.

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

Background References:

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- (6) Gao, Z. et al. (2002) *J. Biol. Chem.* 277, 48115–48121.
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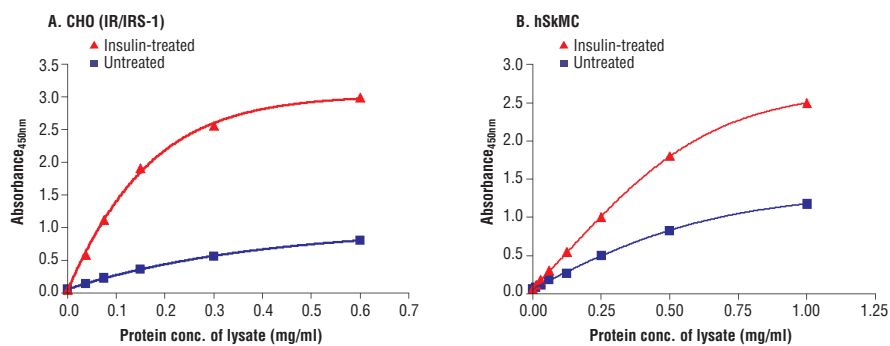


Figure 2. The relationship between lysate protein concentration from untreated and insulin-treated CHO (IR/IRS-1) cells (A) or hSkMC cells (B) and the absorbance at 450 nm is shown.

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 phenylmethyl-sulfonyl 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.

- Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
- Remove media and rinse cells once with ice-cold 1X PBS.
- 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.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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

- Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10⁶ viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
- Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
- Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
- Sonicate lysates on ice.
- 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

- 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.
- 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.
- 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.
- Gently remove the tape and wash wells:
 - Discard plate contents into a receptacle.
 - Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
 - 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.
 - Clean the underside of all wells with a lint-free tissue.
- 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.
- Repeat wash procedure (Section C, Step 4).
- 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.
- Repeat wash procedure (Section C, Step 4).
- 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.
- 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.

- Read results.
 - Visual Determination:** Read within 30 min after adding STOP Solution.
 - Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.