

#7328C Store at 4°C

PathScan® Total IRS-1 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, M, R

Introduction: The PathScan® Total IRS-1 Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of IRS-1. 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, IRS-1 Mouse Detection Antibody* is added to detect 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 absorbance for this developed color is proportional to the quantity of total IRS-1.

* Antibodies in kit are custom formulations specific to kit.

Specificity/Sensitivity: CST's PathScan® Total IRS-1 Sandwich ELISA Kit #7328 detects endogenous levels of IRS-1. 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. However, 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). In Figure 3, Western analysis of protein captured in microwells coated with the IRS-1 antibody shows a major band corresponding to the IRS-1 protein.

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.

Product Includes	Item #	Kit Quantity	Color	Storage Temp
IRS-1 Rabbit mAb Coated Microwells*	92971	96 tests		4°C
IRS-1 Mouse Detection mAb	14088	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.

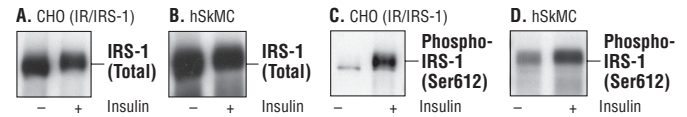
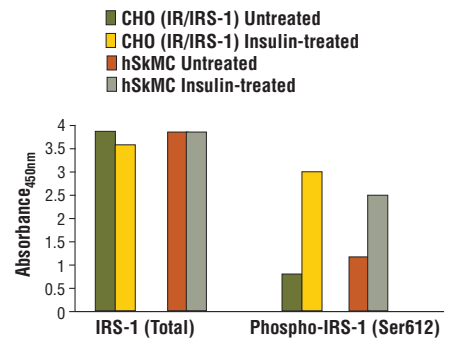


Figure 1. Treatment of hSkMC or CHO (IR/IRS-1) cells with insulin stimulates phosphorylation of IRS-1 at Ser612, detected by the PathScan® Phospho-IRS-1 (Ser612) Sandwich ELISA Kit #7332, but does not affect the level of total IRS-1 protein detected by PathScan® Total IRS-1 Sandwich ELISA Kit #7328. 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.

U.S. Patent No. 5,675,063

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

Background References:

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- (3) Myers Jr., M.G. et al. (1993) *Endocrinology* 132, 1421–1430.
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- (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.

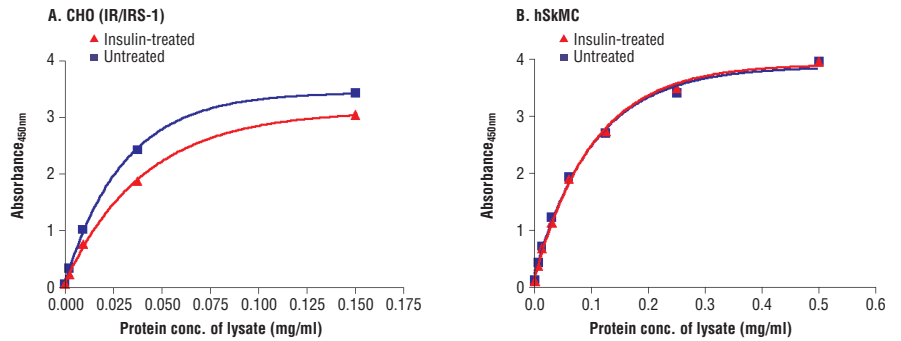


Figure 2. The relationship between lysate protein concentration from untreated and insulin-treated CHO (IR/IRS-1) cells (A) or hSkMC cells (B) and the absorbance at 450 nm is shown.

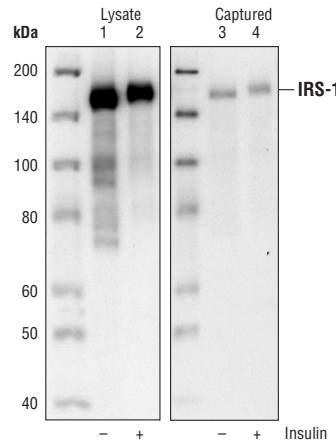


Figure 3. Kit specificity as demonstrated by Western analysis of the ELISA microwell captured protein. Lysates were prepared from CHO (IR/IRS-1) cells and incubated in microwells coated with the IRS-1 capture antibody. Wells were washed, and the captured protein was solubilized in SDS gel loading buffer. Western analysis of CHO (IR/IRS-1) cell starting lysate (lanes 1 & 2) and the captured protein (lanes 3 & 4) was performed using IRS-1 (L3D12) Mouse mAb #3194. The major band detected in the captured material corresponds to IRS-1 (lanes 3 & 4).

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

A Solutions and Reagents

NOTE: Prepare solutions with purified water.

- Microwell strips:** Bring all to room temperature before use.
- 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.
- 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.
- Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 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.
- TMB Substrate** (#7004).
- 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 × 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.