

PathScan® Phospho-IRS1 (panTyr) Sandwich ELISA Antibody Pair

✓ 1 Kit
(4 X 96 assays)

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com

Support ■ 877-678-TECH (8324)
info@cellsignal.com

Web ■ www.cellsignal.com

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

Species Cross-Reactivity: H, R, M

Description: CST's PathScan® Phospho-IRS-1 (panTyr) Sandwich ELISA Antibody Pair is being offered as an economical alternative to our PathScan® Phospho-IRS-1 (panTyr) Sandwich ELISA Kit #7133. Capture and detection antibodies (100X stocks) and an HRP-conjugated secondary antibody (1000X stock) are supplied. Sufficient reagents are supplied for 4 x 96 well ELISAs. The IRS-1 Rabbit Capture Antibody is coated in PBS overnight in a 96 well microplate. After blocking, cell lysate is added followed by a Phospho-Tyrosine Mouse Detection Antibody and an HRP-conjugated, Anti-Mouse IgG Antibody. HRP substrate (TMB) is added for color development. The magnitude of the absorbance for this developed color is proportional to the quantity of IRS-1 phosphorylated on tyrosine.

*Antibodies in this kit are custom formulations specific to the kit.

Reagents not supplied:

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween-20 (PBST-20X) #9809

Cell Lysis Buffer (10X) #9803

TMB Substrate #7004

STOP Solution #7002

Blocking Buffer: 1X PBS/0.05% Tween-20, 1% BSA

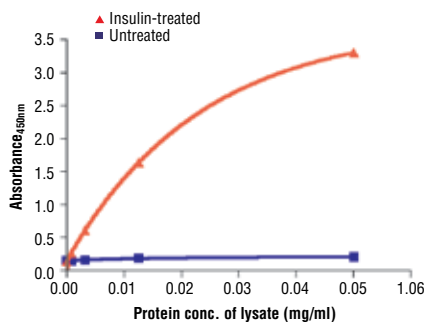
96 Well Microplates**

Microplate Reader

** Antibody Pairs have been validated on Corning® 96 Well Clear Polystyrene High Bind Stripwell™ Microplates (#2592) and Corning® 96 Well EIA/RIA Easy Wash™ Clear Flat Bottom Polystyrene High Bind Microplates (#3369).

Note: Antibody pairs have been optimized using recommended buffers, reagents, plates and the included protocol. Solutions should be made fresh daily.

| Products Included | Volume | Cap Color | Storage |
|--|---------|-----------|---------|
| IRS-1 Rabbit Capture Antibody (100X) | 0.4 ml | Pink | 4°C |
| Phospho-Tyrosine Mouse Detection Antibody (100X) | 0.4 ml | Blue | 4°C |
| Anti-Mouse IgG, HRP-Linked Antibody (1000X) | 0.04 ml | Yellow | -20°C |



The relationship between the protein concentration of the lysate from untreated and insulin-treated CHO (IR/IRS-1) cells and the absorbance at 450 nm using PathScan® Phospho-IRS-1 (panTyr) Sandwich ELISA Antibody Pair #7347 is shown. After overnight starvation, CHO (IR/IRS-1) cells were treated with insulin (100 nM) for 7 minutes at 37°C and then lysed.

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 that 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) while Ser789 is phosphorylated by SIK-2, a member of AMPK family (7). The PKC and mTOR pathways mediate phosphorylation of IRS-1 at Ser612 and Ser636/639, respectively (8,9). Phosphorylation of IRS-1 at Ser1101 is mediated by PKCθ and results in an inhibition of insulin signaling in the cell, which suggests a potential mechanism for insulin resistance in some models of obesity (10).

Storage: IRS-1 Rabbit Capture and Phospho-Tyrosine Mouse Detection Antibodies are stored at 4°C. Anti-Mouse IgG, HRP-Linked Antibody is stored at -20°C.

Companion Products:

PathScan® Phospho-IRS-1 (panTyr) Sandwich ELISA Kit #7133

IRS-1 Antibody #2382

IRS-1 (59G8) Rabbit mAb #2390

IRS-1 (L3D12) Mouse mAb #3194

Phospho-Tyrosine Mouse mAb (P-Tyr-102) #9416

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

Anti-mouse IgG, HRP-linked Antibody #7076

STOP Solution #7002

BSA #9998

Phosphate Buffered Saline (PBS-20X) #9808

Phosphate Buffered Saline with Tween 20 (PBST-20X) #9809

Background References:

- (1) Sun, X.J. et al. (1991) *Nature* 352, 73-77.
- (2) Sun, X.J. et al. (1992) *J. Biol. Chem.* 267, 22662-22672.
- (3) Myers Jr., M.G. et al. (1993) *Endocrinology* 132, 1421-1430.
- (4) Wang, L.M. et al. (1993) *Science* 261, 1591-1594.
- (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

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

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.

PathScan® Sandwich ELISA Antibody Pair Protocol

A Required Reagents

- Coating Buffer:** 1X PBS, (20X PBS #9808)
3.2 mM Na₂HPO₄, 0.5 mM KH₂PO₄, 1.3 mM KCl, 135 mM NaCl, pH 7.4
- Wash Buffer:** 1X PBS/0.05% Tween-20, (20X PBST #9809)
- Blocking Buffer:** 1X PBS/0.05% Tween-20, 1% BSA
- 1X Cell Lysis Buffer:** (10X Cell Lysis Buffer #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 Na₃VO₄, 1 μg/ml leupeptin.
- TMB Substrate:** (TMB Substrate #7004)
- STOP Solution:** (STOP Solution #7002)

NOTE: Reagents should be made fresh daily

B Coating Procedure

- Rinse microplate with dH₂O. Add 200 μl of dH₂O and discard liquid. Blot on paper towel to make sure wells are dry.
- Dilute capture antibody 1:100 in PBS. For a single 96 well plate, add 100 μl of Capture Antibody Stock to 9.9 ml PBS. Mix well and add 100 μl/well. Cover plate and incubate overnight at 4°C (17-20 hours).
- After overnight coating, gently uncover plate and wash wells:**
 - Discard plate contents into a receptacle.
 - Wash 4 times with 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.
- Block plates. Add 150 μl of Blocking Buffer/well, cover plate and incubate at 37°C for 2 hours.
- After blocking, wash plate as in Step 3. Plate is ready to use.

C Preparing Cell Lysates

- Aspirate media, treat cells by adding fresh media containing regulator for desired time.
- To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
- Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM phenyl-methylsulfonyl fluoride (PMSF) to each plate (10 cm²) and incubate the plate on ice for 5 minutes.
- Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
- Sonicate lysates on ice.
- 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.

D Test Procedure

- Lysates can be used undiluted or diluted in Blocking Buffer. 100 μl of lysate is added per well. Cover plate and incubate at 37°C for 2 hours.
- Wash plate as in Coating Procedure, Step 3.
- Dilute detection antibody 1:100 in Blocking Buffer. For a single 96 well plate, add 100 μl of Detector Antibody Stock to 9.9 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover plate and incubate at 37°C for 1 hour.
- Plate is washed as in Coating Procedure, Step 3.
- Secondary antibody, either, anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in Blocking Buffer. For a single 96 well plate, add 10 μl of secondary antibody stock to 9.99 ml of Blocking Buffer. Mix well and add 100 μl/well. Cover and incubate at 37°C for 30 minutes.
- Wash plate as in Coating Procedure, Step 3.
- Add 100 μl of TMB Substrate per well. Cover and incubate at 37°C for 10 minutes.
- Add 100 μl of STOP Solution per well.
- Read plate on a microplate reader at Absorbance 450 nm.