

**PathScan® Phospho-IRS-1 (panTyr)  
Sandwich ELISA Antibody Pair****Orders:** 877-616-CELL (2355)  
orders@cellsignal.com**Support:** 877-678-TECH (8324)**Web:** info@cellsignal.com  
cellsignal.com**Species Cross Reactivity:** H M R  
**UniProt ID:** #P35568  
**Entrez-Gene ID:** #3667

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**For Research Use Only. Not for Use in Diagnostic Procedures.****Description**

CST's PathScan® Phospho-IRS-1 (panTyr) Sandwich ELISA Antibody Pair is 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 kit are custom formulations specific to 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).

**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 the 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, suggesting a potential mechanism for insulin resistance in some models of obesity (10).

**Background References**

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#7347

# PathScan<sup>®</sup> Phospho-IRS-1 (panTyr) Sandwich ELISA Antibody Pair

## ELISA Antibody Pair

### A. Solutions and Reagents

**NOTE:** Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH<sub>2</sub>O, mix.
2. **Wash Buffer:** 1X PBS/0.05% Tween<sup>®</sup> 20, (20X PBST #9809).
3. **Blocking Buffer:** 1X PBS/0.05% Tween<sup>®</sup> 20, 1% BSA.
4. **1X Cell Lysis Buffer:** 10X Cell Lysis Buffer (#9803): To prepare 10 ml of 1X Cell Lysis Buffer, add 1 ml of 10X Cell Lysis Buffer to 9 ml of dH<sub>2</sub>O, mix. Buffer can be stored at 4°C for short-term use (1-2 weeks).

**Recommended:** Add 1 mM phenylmethylsulfonyl fluoride (PMSF) (#8553) immediately before use.

5. **Bovine Serum Albumin (BSA):** (#9998).
6. **TMB Substrate:** (#7004).
7. **STOP Solution:** (#7002)

**NOTE:** Reagents should be made fresh daily.

### 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 (x14,000 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 (~1,200 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 (~1,200 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 (x14,000 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. Coating Procedure

1. Rinse microplate with 200 µl of dH<sub>2</sub>O, discard liquid. Blot on paper towel to make sure wells are dry.
2. Dilute capture antibody 1:100 in 1X PBS. For a single 96 well plate, add 100 µl of capture antibody stock to 9.9 ml 1X PBS. Mix well and add 100 µl/well. Cover plate and incubate overnight at 4°C (17-20 hr).
3. **After overnight coating, gently uncover plate and wash wells:**
  1. Discard plate contents into a receptacle.
  2. Wash four times with wash buffer, 200 µl each time per well. For each wash, strike plates on fresh paper towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
  3. Clean the underside of all wells with a lint-free tissue.
4. Block plates. Add 150 µl of blocking buffer/well, cover plate, and incubate at 37°C for 2 hr.
5. After blocking, wash plate (Section C, Step 3). Plate is ready to use.

### D. Test Procedure

1. Lysates can be used undiluted or diluted in blocking buffer. 100  $\mu$ l of lysate is added per well. Cover plate and incubate at 37°C for 2 hr.
2. Wash plate (Section C, Step 3).
3. Dilute detection antibody 1:100 in blocking buffer. For a single 96 well plate, add 100  $\mu$ l of detection antibody Stock to 9.9 ml of blocking buffer. Mix well and add 100  $\mu$ l/well. Cover plate and incubate at 37°C for 1 hr.
4. Wash plate (Section C, Step 3).
5. Secondary antibody, either streptavidin anti-mouse or anti-rabbit-HRP, is diluted 1:1000 in blocking buffer. For a single 96 well plate, add 10  $\mu$ l of secondary antibody stock to 9.99 ml of blocking buffer. Mix well and add 100  $\mu$ l/well. Cover and incubate at 37°C for 30 min.
6. Wash plate (Section C, Step 3).
7. Add 100  $\mu$ l of TMB substrate per well. Cover and incubate at 37°C for 10 min.
8. Add 100  $\mu$ l of STOP solution per well. Shake gently for a few seconds.
9. Read plate on a microplate reader at absorbance 450 nm.
  1. **Visual Determination:** Read within 30 min after adding STOP solution.
  2. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP solution.

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