

**#2580** Store at -20°C

# Phospho-IRS-1 (Ser332/336) Antibody

100 µl  
 (10 Western mini-blot)



**Orders** ■ 877-616-CELL (2355)  
 orders@cellsignaling.com  
**Support** ■ 877-678-TECH (8324)  
 info@cellsignaling.com  
**Web** ■ www.cellsignaling.com

rev. 09/22/08

This product is for *in vitro* research use only and is not intended for use in humans or animals.  
 This product is not intended for use as a therapeutic or in diagnostic procedures.

Applications W Transfected	Species Cross-Reactivity* R, (H, M)	Molecular Wt. 180 kDa	Source Rabbit**
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**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.

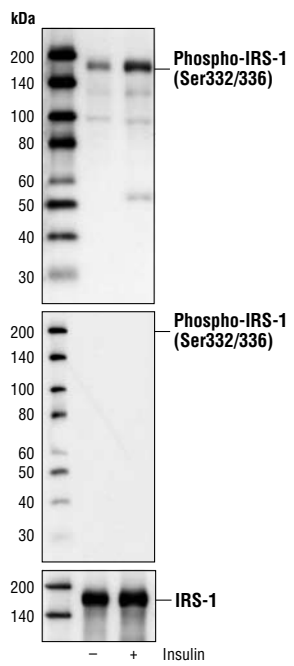
GSK-3-mediated IRS-1 serine phosphorylation leads to inhibition of insulin-stimulated IRS-1 signaling. Ser332 and Ser336 of IRS-1 are situated in a glycogen synthase kinase-3 (GSK-3) consensus motif (SXXXS), and it has been shown that Ser332 is the actual GSK-3 phosphorylation site while Ser336 provides a "priming" site necessary for GSK-3 action (11).

**Specificity/Sensitivity:** Phospho-IRS-1 (Ser332/336) Antibody detects transfected levels of IRS-1 when phosphorylated at Ser332/336. It also detects IRS-1 protein when singly phosphorylated at Ser332 or Ser336 of human IRS-1. This antibody does not cross-react with other related phosphoproteins.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Ser332/336 of mouse IRS-1 (equivalent to Ser337/341 of human IRS-1). Antibodies are purified by peptide affinity chromatography.

**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.



*Western blot analysis of cell extracts from CHO cells overexpressing insulin receptor and IRS-1, untreated or treated with insulin, using Phospho-IRS-1 (Ser332/336) Antibody (upper and middle) or IRS-1 Antibody #2382 (lower). The middle blot was treated with calf intestinal phosphatase (CIP) before antibody probing.*

- (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.
- (11) Liberman, Z. and Eldar-Finkelman, H. (2005) *J. Biol. Chem.* 280, 4422-4428.

**Entrez-Gene ID #** 3667  
**Swiss-Prot Acc. #** P35568

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at -20°C. Do not aliquot the antibody.

**\*Species cross-reactivity is determined by Western blot.**  
**\*\*Anti-rabbit secondary antibodies must be used to detect this antibody.**

**Recommended Antibody Dilutions:**  
 Western blotting 1:1000

- Companion Products:**
- Phospho-IRS-1 (Ser302) Antibody #2384
  - Phospho-IRS-1 (Ser302) (34C7) Rabbit mAb #2491
  - Phospho-IRS-1 (Ser307) Antibody #2381
  - Phospho-IRS-1 (Ser612) Antibody #2386
  - Phospho-IRS-1 (Ser612) (L7B8) Mouse mAb #3193
  - Phospho-IRS-1 (Ser636/639) Antibody #2388
  - Phospho-IRS-1 (Ser789) Antibody #2389
  - Phospho-IRS-1 (Ser1101) Antibody #2385
  - IRS-1 Antibody #2382
  - IRS-1 (L3D12) Mouse mAb #3194
  - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
  - Anti-rabbit IgG, HRP-linked Antibody #7074
  - Prestained Protein Marker, Broad Range (Premixed Format) #7720
  - Biotinylated Protein Ladder Detection Pack #7727
  - 20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope®-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO® substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.