

Phospho-IRS-1 (Ser612) (C15H5) Rabbit mAb

- Small 100 μ l
(10 Western mini-blot)
- Petite 40 μ l
(4 Western mini-blot)

New 02/08

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

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

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

Web ■ www.cellsignal.com

Applications	Species Cross-Reactivity*	Molecular Wt.	Source	Isotype
W Endogenous	H, M, R	180 kDa	Rabbit**	IgG

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 PKC θ 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).

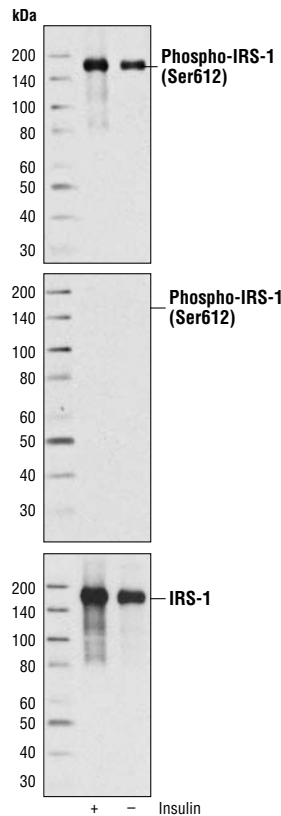
Phosphorylation of IRS-1 at Ser612 by MAPK downregulates insulin signaling and may be part of a response to high glucose/glucosamine levels (11).

Specificity/Sensitivity: Phospho-IRS-1 (Ser612) (C15H5) Rabbit mAb detects endogenous levels of IRS-1 only when phosphorylated at Ser612.

Source/Purification: Monoclonal antibody is produced by immunizing rabbits with a synthetic phosphopeptide (KLH-coupled) corresponding to residues surrounding Ser612 of mouse IRS-1.

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.
- (11) Andreozzi, F. et al. (2004) *Endocrinology* 145, 2845-57.



Western blot analysis of cell extracts from CHO IR/IRS-1 cells, untreated or treated with insulin, using Phospho-IRS-1 (Ser612) (C15H5) Rabbit mAb (upper and middle) or IRS-1 Antibody #2382 (lower). The middle blot was treated with calf intestinal phosphatase (CIP) before antibody probing.

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

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 μ g/ml BSA, 50% glycerol and less than 0.02% sodium azide. 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 (Ser332/336) Antibody #2580
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 #7727

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.

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry IC—Immunocytochemistry IF—Immunofluorescence
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken X—Xenopus
Species enclosed in parentheses are predicted to react based on 100% sequence homology.

F—Flow cytometry E—ELISA D—DELFIATM
Z—zebra fish B—bovine All—all species expected

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 cm²) 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.