

#3015 Store at -20°C

Insulin Receptor Substrate Antibody Sampler Kit

✓ 1 Kit
(5 x 40 µl)



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

rev. 06/09/11

This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-IRS-1 (Ser307) Antibody	2381	40 µl	180 kDa	Rabbit
Phospho-IRS-1 (Ser612) (C15H5) Rabbit mAb	3203	40 µl	180 kDa	Rabbit
Phospho-IRS-1 (Ser318) (D51C3) Rabbit mAb	5610	40 µl	180 kDa	Rabbit
IRS-1 (D23G12) Rabbit mAb	3407	40 µl	180 kDa	Rabbit
IRS-2 Antibody	4502	40 µl	180 kDa	Rabbit
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

Description: The Insulin Receptor Substrate Antibody Sampler Kit provides an economical means to investigate IRS-1 and IRS-2 signaling and phosphorylation within the cell. The kit contains primary and secondary antibodies to perform four western blots with each antibody.

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).

Specificity/Sensitivity: Phospho-IRS-1 (Ser307) Antibody, Phospho-IRS-1 (Ser612) (C15H5) Rabbit mAb and Phospho-IRS-1 (Ser636/639) Antibody detect endogenous levels of IRS-1 only when phosphorylated at Ser307, Ser612 or Ser636/639, respectively. IRS-1 Antibody and IRS-2 Antibody detect endogenous levels of total IRS-1 or IRS-2, respectively. None of the antibodies in this kit cross-react with any related proteins

Source/Purification: Polyclonal antibodies are produced by immunizing animals with a synthetic peptide corresponding to the carboxy-terminal sequence of human IRS-2, residues surrounding Ser307 of mouse IRS-1 (homologous to Ser312 of human IRS-1), and residues surrounding serine 636/639 of human IRS-1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser612 of mouse IRS-1 and a synthetic peptide corresponding to the sequence surrounding Ser270 of human IRS-1.

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 antibodies.

Recommended Antibody Dilutions:

Western blotting 1:1000

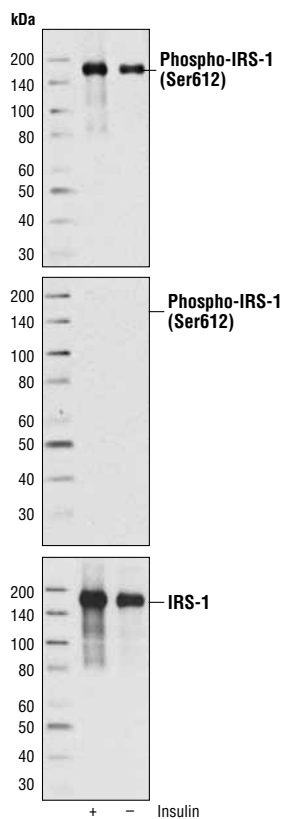
Please visit www.cellsignal.com for a complete listing of recommended companion products.

Background References:

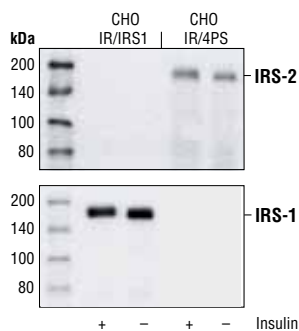
- (1) Sun, X.J. et al. (1991) *Nature* 352, 73-7.
- (2) Sun, X.J. et al. (1992) *J Biol Chem* 267, 22662-72.
- (3) Myers, M.G. et al. (1993) *Endocrinology* 132, 1421-30.
- (4) Wang, L.M. et al. (1993) *Science* 261, 1591-4.
- (5) Rui, L. et al. (2001) *J Clin Invest* 107, 181-9.
- (6) Gao, Z. et al. (2002) *J Biol Chem* 277, 48115-21.
- (7) Horike, N. et al. (2003) *J Biol Chem* 278, 18440-7.
- (8) Ozes, O.N. et al. (2001) *Proc Natl Acad Sci U S A* 98, 4640-5.
- (9) De Fea, K. and Roth, R.A. (1997) *Biochemistry* 36, 12939-47.
- (10) Li, Y. et al. (2004) *J Biol Chem* 279, 45304-7.

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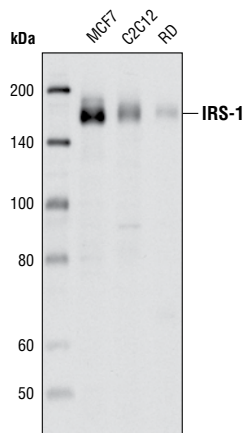
Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebra fish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



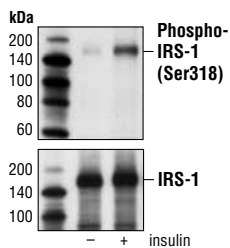
Western blot analysis of cell extracts from CHO IR/IRS-1 cells, untreated or treated with insulin, using **Phospho-IRS-1 (Ser612) (C15H5) Rabbit mAb #3203** (upper and middle) or **IRS-1 Antibody #2382** (lower). The middle blot was treated with calf intestinal phosphatase (CIP) before antibody probing.



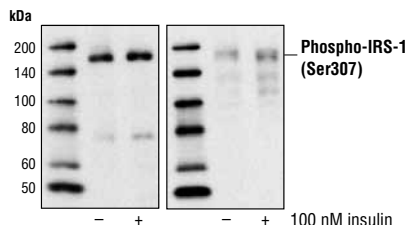
Western blot analysis of extracts from CHO IR/4PS cells (transfected with insulin receptor and IRS-2) or CHO IR/IRS-1 cells (transfected with insulin receptor and IRS-1), showing no change in total protein levels with insulin stimulation (100 nM for 5 min), using **IRS-2 Antibody #4502** (upper) or **IRS-1 Antibody #2382** (lower).



Western blot analysis of MCF7, C2C12 and RD cell lines using **IRS-1 (D23G12) Rabbit mAb #3407**.



Western blot analysis of extracts from serum-starved C2C12 cells, untreated or insulin-treated (150 nM for 5 min.), using **Phospho-IRS-1 (Ser318) (D51C3) Rabbit mAb #5610** (upper), or **IRS-1 Antibody #3407** (lower).



Western blot analysis of extracts from MCF-7 cells, unstimulated or insulin-stimulated (100 nM for 5 min), using **IRS-1 Antibody #2382** (left) and **Phospho-IRS-1 (Ser307) Antibody #2381** (right).

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.