#12879 Store at -20°C

# IRS-1 Inhibition Antibody Sampler Kit

☑ 1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

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

rev. 06/16

**For Research Use Only. Not For Use In Diagnostic Procedures.**

| Products Included | Product # | Quantity | Mol. Wt. | Isotype |
|---|---|---|---|---|
| Phospho-IRS-1 (Ser302) (34C7) Rabbit mAb | 2491 | 20 µl | 180 kDa | Rabbit IgG |
| Phospho-IRS-1 (Ser307) Antibody | 2381 | 20 µl | 180 kDa | Rabbit IgG |
| Phospho-IRS-1 (Ser318) (D51C3) Rabbit mAb | 5610 | 20 µl | 180 kDa | Rabbit IgG |
| Phospho-IRS-1 (Ser612) (C15H5) Rabbit mAb | 3203 | 20 µl | 180 kDa | Rabbit IgG |
| Phospho-IRS-1 (Ser636/639) Antibody | 2388 | 20 µl | 180 kDa | Rabbit IgG |
| Phospho-IRS-1 (Ser1101) Antibody | 2385 | 20 µl | 180 kDa | Rabbit IgG |
| IRS-1 (D23G12) Rabbit mAb | 3407 | 20 µl | 180 kDa | Rabbit IgG |
| 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.**

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

**Recommended Antibody Dilutions:**

| | |
|---|---|
| Western blotting | 1:1000 |

**Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.**

**Description:** The IRS-1 Inhibition Antibody Sampler Kit provides an economical means to evaluate insulin signaling negative feedback loops via phosphorylation of various IRS-1 serine residues. The kit includes enough antibody to perform two western blot experiments with each primary 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, many of which are related to negative feedback loops activated during insulin signaling. Ser302 (human Ser307) of IRS-1 is regulated by FOX01 (5), IKKγ, and MYO1C (6). Ser307 (human Ser312) of IRS-1 is phosphorylated by JNK (7) and IKK (8). PKC phosphorylates mouse IRS-1 at Ser318 (human Ser323) by insulin receptor activation or by other stimulation such as TPA, IL-6, and retinoic acid treatment (9-12). The PKC and mTOR pathways mediate phosphorylation of IRS-1 at Ser612 (human Ser616) and Ser632/635 (human Ser636/639), respectively (13,14). Phosphorylation of IRS-1 at Ser1097 (human 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 (15).

**Specificity/Sensitivity:** Each activation state antibody recognizes the phosphorylated form of its target. All target residues are based on the sequence for mouse IRS-1, except Ser636/339 and Ser1101, which are based on the sequence for human IRS-1. IRS-1 (D23G12) Rabbit mAb recognizes total IRS-1 protein independent of its phosphorylation state.

**Source/Purification:** Polyclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding mouse Ser307 (human Ser312), human Ser636/639 (mouse Ser632/635), and human Ser1101 (mouse Ser1097) of IRS-1. Polyclonal antibodies are purified by protein A and peptide affinity chromatography. Monoclonal antibodies are produced by immunizing animals with synthetic phosphopeptides corresponding to residues surrounding mouse Ser302 (human Ser307), mouse Ser318 (human Ser323), and mouse Ser612 (human 616) of IRS-1. IRS-1 (D23G12) rabbit monoclonal antibody is produced by immunizing animals with a synthetic peptide corresponding to the sequence surrounding Ser270 of human IRS-1 (mouse 1097).

**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) Cao, Y. et al. (2006) *J Biol Chem* 281, 40242-51.
(6) Nakamori, Y. et al. (2006) *J Cell Biol* 173, 665-71.
(7) Rui, L. et al. (2001) *J Clin Invest* 107, 181-9.
(8) Gao, Z. et al. (2002) *J Biol Chem* 277, 48115-21.
(9) Greene, M.W. et al. (2004) *Biochem J* 378, 105-16.
(10) Weigert, C. et al. (2006) *J Biol Chem* 281, 7060-7.
(11) del Rincón, S.V. et al. (2004) *Oncogene* 23, 9269-79.
(12) Moeschel, K. et al. (2004) *J Biol Chem* 279, 25157-63.
(13) Ozes, O.N. et al. (2001) *Proc Natl Acad Sci USA* 98, 4640-5.
(14) De Fea, K. and Roth, R.A. (1997) *Biochemistry* 36, 12939-47.
(15) Li, Y. et al. (2004) *J Biol Chem* 279, 45304-7.

U.S. Patent No. 5,675,063



**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**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

#12879

# Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
**NOTE:** Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

## 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 $dH_2O$, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml $dH_2O$, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with $dH_2O$.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml $dH_2O$, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml $dH_2O$, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml $dH_2O$, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

## B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

## C. Membrane Blocking and Antibody Incubations

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

### I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

### II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

## D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.