Insulin Receptor Substrate Antibody Sampler Kit

- **1 Kit** (5 x 20 µl)

**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 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. 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.

**Background References:**

**Applications Key:**
- **W**—Western
- IP—Immunoprecipitation
- **IIHC**—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
- **Mm**—monkey
- **Mk**—mink
- **C**—chicken
- **Dm**—D. melanogaster
- **X**—Xenopus
- **Z**—zebra fish
- **B**—bovine
- **Dg**—Dog
- **Pg**—Pig
- Sc—*S. cerevisiae*
- **All**—all species expected

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

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**Background References:**

**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 validation data and complete listing of recommended companion products.

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

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

**Web**
- [www.cellsignal.com](http://www.cellsignal.com)
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.

1. 1X Phosphate Buffered Saline (PBS)
2. 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
3. Transfer Buffer: 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. 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).
5. Nonfat Dry Milk (weight to volume [w/v])
6. 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%).
7. Wash Buffer: 1X TBS, 0.1% Tween®20 (TBS/T)
8. Bovine Serum Albumin (BSA)
9. 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%).
10. 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.
11. Prestained Protein Marker, Broad Range (Premixed Format) #7720
12. Biotinylated Protein Ladder Detection Pack #7727
13. 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.

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 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.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. 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.

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.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. 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.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. 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.

2. 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.