

# p-IRS-1 (Ser 636): sc-33957

## BACKGROUND

Insulin receptor substrate-1 (IRS-1) is a substrate of the Insulin receptor that undergoes phosphorylation in response to Insulin, IGF-1 and IL-4. Tyrosine (Tyr) phosphorylation of IRS-1 mediates Insulin-stimulated responses, while Serine (Ser)/Threonine (Thr) phosphorylation of IRS-1 can either enhance or negate Insulin effects. Tyrosines 465, 612, 632, 662, 941 and 989 of IRS-1 resemble YXXM motifs that upon phosphorylation are predicted to bind SH2 domains in the p85 regulatory subunit of PI 3-K, resulting in activation of p110 catalytic subunit. SHP-2 binding to IRS-1 can occur upon phosphorylation at Tyr 1179 and Tyr 1229. GRB2 binding can occur upon phosphorylation at Tyr 896. Rodent Ser 99 and Thr 502 of IRS-1 are casein kinase II-dependent phosphorylation sites. There is an increase in Ser 636 phosphorylation of IRS-1 in primary skeletal muscle cells from patients with type 2 diabetes. IGF-1 and Anisomycin treatment converge downstream onto mTOR and PKC  $\delta$  to induce IRS-1 Ser 312 phosphorylation. Insulin resistance in the aorta of hypertensive rats is associated with elevated IRS-1 phosphorylation at Ser 307 and increased SAPK/JNK activation. IRS-1 contains three putative binding sites for 14-3-3 protein at Ser 270, Ser 374 and Ser 641 that are capable of phosphorylation.

## REFERENCES

- Ogihara, T., et al. 1997. 14-3-3 protein binds to Insulin receptor substrate-1, one of the binding sites of which is in the phosphotyrosine binding domain. *J. Biol. Chem.* 272: 25267-25274.
- Espósito, D.L., et al. 2001. Tyr 612 and Tyr 632 in human insulin receptor substrate-1 are important for full activation of insulin-stimulated phosphatidylinositol 3-kinase activity and translocation of Glut4 in adipose cells. *Endocrinology* 142: 2833-2840.

## CHROMOSOMAL LOCATION

Genetic locus: IRS1 (human) mapping to 2q36.3; Irs1 (mouse) mapping to 1 C5.

## SOURCE

p-IRS-1 (Ser 636) is a rabbit polyclonal antibody raised against a short amino acid sequence containing Ser 636 phosphorylated IRS-1 of human origin.

## PRODUCT

Each vial contains 200  $\mu$ g IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-33957 P, (100  $\mu$ g peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

## STORAGE

Store at 4° C, **\*\*DO NOT FREEZE\*\***. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

p-IRS-1 (Ser 636) is recommended for detection of Ser 636 phosphorylated IRS-1 of mouse, rat and human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2  $\mu$ g per 100-500  $\mu$ g of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500), immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

p-IRS-1 (Ser 636) is also recommended for detection of correspondingly phosphorylated IRS-1 in additional species, including equine, canine, bovine, porcine and avian.

Suitable for use as control antibody for IRS-1 siRNA (h): sc-29376, IRS-1 siRNA (m): sc-29377, IRS-1 shRNA Plasmid (h): sc-29376-SH, IRS-1 shRNA Plasmid (m): sc-29377-SH, IRS-1 shRNA (h) Lentiviral Particles: sc-29376-V and IRS-1 shRNA (m) Lentiviral Particles: sc-29377-V.

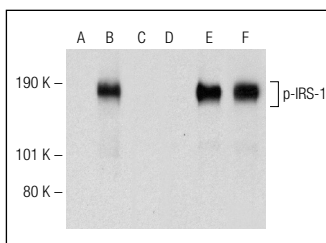
Molecular Weight of p-IRS-1: 170-185 kDa.

Positive Controls: MCF7 + insulin cell lysate: sc-24733, IRS-1 (h): 293T Lysate: sc-116569 or A549 cell lysate: sc-2413.

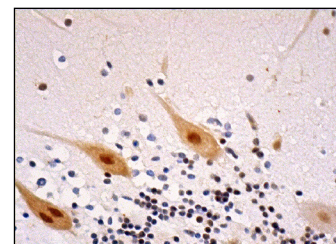
## RECOMMENDED SECONDARY REAGENTS

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto B Blocking Reagent: sc-2335 (use 50 mM NaF, sc-24988, as diluent), Western Blotting Luminol Reagent: sc-2048 and Lambda Phosphatase: sc-200312A. 2) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941. 4) Immunohistochemistry: use ImmunoCruz™: sc-2051 or ABC: sc-2018 rabbit IgG Staining Systems.

## DATA



Western blot analysis of IRS-1 phosphorylation in non-transfected: sc-117752 (A,D), untreated human IRS-1 transfected: sc-116569 (B,E) and lambda protein phosphatase (sc-200312A) treated human IRS-1 transfected: sc-116569 (C,F) 293T whole cell lysates. Antibodies tested include p-IRS-1 (Ser 636): sc-33957 (A,B,C) and IRS-1 (A-19): sc-560 (D,E,F).



p-IRS-1 (Ser 636): sc-33957. Immunoperoxidase staining of formalin fixed, paraffin-embedded human cerebellum tissue showing cytoplasmic and nuclear staining of Purkinje cells and nuclear staining of cells in granular layer and cells in molecular layer.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.