SANTA CRUZ BIOTECHNOLOGY, INC.

p-IRS-1 (Ser 641)-R: sc-22300-R

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 PI3K, 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 phsophorylation 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-I and anisomycin treatment converge downstream onto mTOR and PKC δ 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

1. 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.
2. Esposito, 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.
3. Hers, I., et al. 2002. Reciprocal feedback regulation of Insulin receptor and Insulin receptor substrate tyrosine phosphorylation by phosphoinositide 3-kinase in primary adipocytes. Biochem. J. 368: 875-884.
4. Ishizuka, T., et al. 2004. Protein kinase C (PKC) beta modulates Serine phosphorylation of Insulin receptor substrate-1 (IRS-1)-effect of overexpression of PKCβ on Insulin signal transduction. Endocr. Res. 30: 287-299.
5. Liu, Y.F., et al. 2004. Serine phosphorylation proximal to its phosphotyrosine binding domain inhibits Insulin receptor substrate-1 function and promotes Insulin resistance. Mol. Cell Biol. 24: 9668-9681.

CHROMOSOMAL LOCATION

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

SOURCE

p-IRS-1 (Ser 641)-R is a rabbit polyclonal antibody raised against a short amino acid sequence containing phosphorylated Ser 641 of IRS-1 of human origin.

PRODUCT

Each vial contains 200 µg IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

Blocking peptide available for competition studies, sc-22300 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

p-IRS-1 (Ser 641)-R is recommended for detection of Ser 641 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 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and solid phase ELISA (starting dilution 1:30, dilution range 1:30-1:3000).

Suitable for use as control antibody for IRS-1 siRNA (h): sc-29376 and IRS-1 siRNA (m): sc-29377.

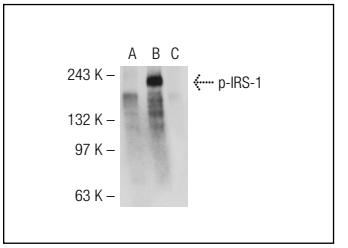
Molecular Weight of p-IRS-1: 170-185 kDa.

Positive Controls: MCF7 + Insulin cell lysate: sc-24733, NIH/3T3 whole cell lysate: sc-2210 or NIH/3T3 + anisomycin cell lysate: sc-2247.

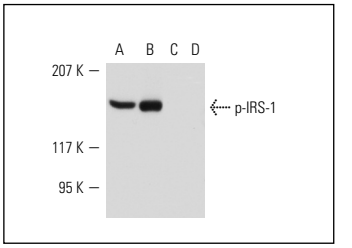
RECOMMENDED SECONDARY REAGENTS

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.

DATA



p-IRS-1 (Ser 641)-R: sc-22300-R. Western blot analysis of IRS-1 phosphorylation in non-transfected: sc-117752 (**A**), untreated human IRS-1 transfected: sc-116569 (**B**) and lambda protein phosphatase (sc-200312A) treated human IRS-1 transfected: sc-116569 (**C**) 293T whole cell lysates.



p-IRS-1 (Ser 641)-R: sc-22300-R. Western blot analysis of IRS-1 phosphorylation in untreated (**A**,**C**) and anisomycin treated (**B**,**D**) NIH/3T3 whole cell lysates. Antibody was pre-incubated with cognate phosphorylated peptide in blots (**C**) and (**D**).

STORAGE

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

RESEARCH USE

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