SANTA CRUZ BIOTECHNOLOGY, INC.

p-IRS-1 (Tyr 465): sc-17194

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Insulin receptor substrate-1 (IRS-1) is a 170-185 kDa 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) β modulates Serine phosphorylation of Insulin receptor substrate-1 (IRS-1)-effect of overexpression of PKCβ on Insulin signal transduction. Endocr. Res. 30: 287-299.

CHROMOSOMAL LOCATION

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

SOURCE

p-IRS-1 (Tyr 465) is available as either goat (sc-17194) or rabbit (sc-17194-R) polyclonal affinity purified antibody raised against a short amino acid sequence containing Tyr 465 phosphorylated 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-17194 P, (100 µg peptide in 0.5 ml PBS containing < 0.1% sodium azide and 0.2% BSA).

APPLICATIONS

p-IRS-1 (Tyr 465) is recommended for detection of Tyr 465 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), 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).

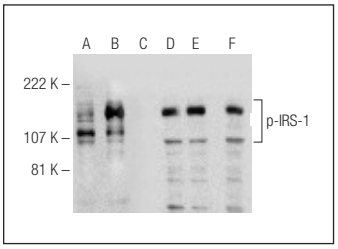
p-IRS-1 (Tyr 465) is also recommended for detection of correspondingly phosphorylated IRS-1 in additional species, including equine, canine, bovine and porcine.

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.

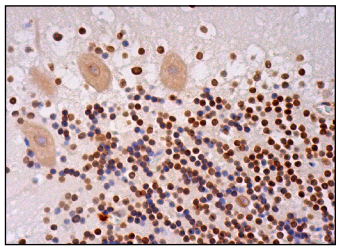
Molecular Weight of p-IRS-1: 170-185 kDa.

Positive Controls: MCF7 + Insulin cell lysate: sc-24733 or A549 cell lysate: sc-2413.

DATA



Western blot analysis of IRS-1 phosphorylation in untreated (**A**,**D**), insulin treated (**B**,**E**) and insulin and lambda protein phosphatase (sc-200312A) treated (**C**,**F**) MCF7 whole cell lysates. Antibodies tested include p-IRS-1 (Tyr 465)-R: sc-17194-R (**A**,**B**,**C**) and IRS-1 (A-19): sc-560 (**D**,**E**,**F**).



p-IRS-1 (Tyr 465)-R: sc-17194-R. 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.

SELECT PRODUCT CITATIONS

1. Moore, T., et al. 2008. Reduced susceptibility to two-stage skin carcinogenesis in mice with low circulating Insulin-like growth factor I levels. Cancer Res. 68: 3680-3688.

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.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.