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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

SANTA CRUZ BIOTECHNOLOGY, INC.

IRS-1 shRNA (h2) Lentiviral Particles: sc-44268-V

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

The Insulin receptor substrate-1 (IRS-1), a protein major substrate of the Insulin receptor, is phosphorylated in response to stimulation of cells by Insulin, Insulin-like growth factor 1 (IGF-1) and interleukin 4 (IL-4). IRS-1 is phosphorylated on serine, threonine and tyrosine residues in a variety of tissues. An Insulin-sensitive serine/threonine kinase casein kinase II mediates a portion of the Insulin-stimulated serine/threonine phosphorylation of overexpressed IRS-1 *in vivo*. Thr 502 is identified as the major casein kinase II-catalyzed phosphorylation site in rat IRS-1, and Ser 99 is an additional phosphorylation site catalyzed by casein kinase II. Thus, casein kinase II-catalyzed phosphorylation of IRS-1 may be a component of the intracellular Insulin signaling cascade. IRS-1 contains three putative binding sites for 14-3-3 (Ser 270, Ser 374 and Ser 641) and the motif around Ser 270 is located in the phosphotyrosine binding domain of IRS-1, which is responsible for the interaction with the Insulin receptor. The association of 14-3-3 with IRS-1 increases significantly upon treatment with okadaic acid, a potent serine/ threonine phosphatase inhibitor. Therefore, the association of 14-3-3 protein may play a role in the regulation of Insulin sensitivity by interrupting the association between the Insulin receptor and IRS-1.

REFERENCES

1. Myers, M.G., et al. 1992. IRS-1 activates the phosphatidylinositol 3'-kinase by associating with the src homology 2 domains of p85. Proc. Natl. Acad. Sci. USA 89: 10350-10354.
2. Myers, M.G., et al. 1993. IRS-1 is a common element in Insulin and IGF signaling to the phosphatidylinositol 3'-kinase. Endocrinology 132: 1421-1430.
3. Myers, M.G., et al. 1993. The new elements of Insulin signaling: Insulin receptor substrate-1 and proteins with SH2 domains. Diabetes 42: 643-650.
4. Tanasijevic, M.J., et al. 1993. Phosphorylation of the Insulin receptor substrate IRS-1 by casein kinase II. J. Biol. Chem. 268: 18157-18166.
5. 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.

CHROMOSOMAL LOCATION

Genetic locus: IRS1 (human) mapping to 2q36.3.

PRODUCT

IRS-1 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see IRS-1 siRNA (h2): sc-44268 and IRS-1 shRNA Plasmid (h2): sc-44268-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

IRS-1 shRNA (h2) Lentiviral Particles is recommended for the inhibition of IRS-1 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

IRS-1 (C-20): sc-559 is recommended as a control antibody for monitoring of gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

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 A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor IRS-1 gene expression knockdown using RT-PCR Primer: IRS-1 (h2)-PR: sc-44268-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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