

SIRP- α siRNA (r): sc-270499

BACKGROUND

SIRPs (signal-regulatory proteins) are a family of transmembrane glycoproteins that were identified by their association with the Src homology 2 domain-containing protein-tyrosine phosphatase SHP-2 in response to Insulin. The SIRP family negatively regulates the PI 3-kinase pathway, which may diminish EGFR-mediated motility and survival phenotypes that contribute to transformation of certain cell types. SIRP- α 1 is a transmembrane protein which contains an extracellular portion with three immunoglobulin-like structures and a cytoplasmic region with four potential tyrosine phosphorylation sites. SIRP- α (also known as SIRP- α 1, SIRP- α 2 or SIRP- α 3) is a substrate for activated receptor tyrosine kinases. In its tyrosine phosphorylated form, SIRP- α binds to SH-PTP2 through SH2 interactions and acts as an SH-PTP2 substrate. SIRP- α has been shown to have negative regulatory effects on cellular responses induced by growth factors, oncogenes and Insulin. SIRP- β 1 shares extensive sequence homology with SIRP- α in its extracellular portion but lacks the cytoplasmic portion. SIRP- γ , originally designated SIRP- β 2 (SIRP-B2, CD172 γ) has unique characteristics from both the α and β versions. SIRP- γ is expressed on the majority of T cells and a proportion of B cells. CD47 associates with SIRP- γ , and this interaction signals unidirectionally only.

REFERENCES

1. Yamauchi, K., et al. 1995. Identification of the major SHPTP2-binding protein that is tyrosine-phosphorylated in response to Insulin. *J. Biol. Chem.* 270: 17716-17722.
2. Fujioka, Y., et al. 1996. A novel membrane glycoprotein, SHPS-1, that binds the SH2-domain-containing tyrosine phosphatase SHP-2 in response to mitogens and cell adhesion. *Mol. Cell. Biol.* 16: 6887-6899.
3. Kharitonov, A., et al. 1997. A family of proteins that inhibit signalling through tyrosine kinase receptors. *Nature* 386: 181-186.
4. Stofega, M.R., et al. 1998. Growth hormone regulation of SIRP and SHP-2 tyrosyl phosphorylation and association. *J. Biol. Chem.* 273: 7112-7117.
5. Wu, C.J., et al. 2000. Inhibition of EGFR-mediated phosphoinositide-3-OH kinase (PI-3 K) signaling and glioblastoma phenotype by signal-regulatory proteins (SIRPs). *Oncogene* 19: 3999-4010.

CHROMOSOMAL LOCATION

Genetic locus: Sirpa (rat) mapping to 3q36.

PRODUCT

SIRP- α siRNA (r) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SIRP- α shRNA Plasmid (r): sc-270499-SH and SIRP- α shRNA (r) Lentiviral Particles: sc-270499-V as alternate gene silencing products.

For independent verification of SIRP- α (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270499A, sc-270499B and sc-270499C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SIRP- α siRNA (r) is recommended for the inhibition of SIRP- α expression in rat cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

SIRP- α / β (A-1): sc-17803 is recommended as a control antibody for monitoring of SIRP- α 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SIRP- α gene expression knockdown using RT-PCR Primer: SIRP- α (r)-PR: sc-270499-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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

PROTOCOLS

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