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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](https://www.linkedin.com/company/szaboscandic) 

SIRP- α shRNA (h) Lentiviral Particles: sc-44106-V

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-K pathway, which may diminish EGFR-mediated motility and survival phenotypes that contribute to transformation of certain cell types. SIRP- α 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 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 1 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, CD172g) 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.

CHROMOSOMAL LOCATION

Genetic locus: SIRPA (human) mapping to 20p13.

PRODUCT

SIRP- α shRNA (h) 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 μ l frozen stock containing 1.0×10^6 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 SIRP- α siRNA (h): sc-44106 and SIRP- α shRNA Plasmid (h): sc-44106-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.

PROTOCOLS

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

APPLICATIONS

SIRP- α shRNA (h) Lentiviral Particles is recommended for the inhibition of SIRP- α expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

SIRP- α (C-7): sc-376884 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SIRP- α gene expression knockdown using RT-PCR Primer: SIRP- α (h)-PR: sc-44106-PR (20 μ l, 591 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.