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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) 

c-Src shRNA (h2) Lentiviral Particles: sc-44250-V

BACKGROUND

The major translational products of the Src gene family are membrane-associated tyrosine protein kinases that lack transmembrane and external amino acid sequences. By virtue of their common structural motifs, the Src family is composed of nine members in vertebrates, including c-Src, c-Yes, Fgr, Yrk, Fyn, Lyn, Hck, Lck and Blk. Src family kinases, which contain an amino-terminal cell membrane anchor followed by SH3 and SH2 domains, transduce signals that are involved in the control of a variety of cellular processes, including proliferation, differentiation, motility and adhesion. Src family members are normally maintained in an inactive state and can be activated transiently during cellular events such as mitosis. Different subcellular locations of Src family kinases may be important for the regulation of specific cellular processes, such as mitogenesis, cytoskeletal organization and membrane trafficking. c-Src (also designated pp60Src, Src p60 and proto-oncogene tyrosine protein kinase Src) is expressed in a broad range of tissue and cell types, although the highest levels of c-Src are detected in neuronal tissues and platelets. c-Src may play a role in events associated with both neuronal differentiation and maintenance of mature neuronal cell functions.

REFERENCES

1. Sakaguchi, A.Y., et al. 1982. Organization of human proto-oncogenes. *Am. J. Hum. Genet.* 34: 175.
2. Brugge, J.S., et al. 1985. Neurons express high levels of structurally modified, activated form of pp60Src. *Nature* 316: 554-557.
3. Golden, A., et al. 1986. Bloodplatelets express high levels of the pp60c-src-specific tyrosine kinase activity. *Proc. Natl. Acad. Sci. USA* 83: 852-856.
4. Cartwright, C.A., et al. 1987. Alterations in pp60Src accompany differentiation of neurons from rat embryo striatum. *Mol. Cell. Biol.* 7: 1830-1840.
5. Wiestler, O.D., et al. 1988. Developmental expression of two forms of pp60Src in mouse brain. *Mol. Cell. Biol.* 8: 502-504.
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CHROMOSOMAL LOCATION

Genetic locus: SRC (human) mapping to 20q11.23.

PRODUCT

c-Src 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 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 c-Src siRNA (h2): sc-44250 and c-Src shRNA Plasmid (h2): sc-44250-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

c-Src shRNA (h2) Lentiviral Particles is recommended for the inhibition of c-Src 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

c-Src (H-12): sc-5266 is recommended as a control antibody for monitoring of c-Src 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 c-Src gene expression knockdown using RT-PCR Primer: c-Src (h2)-PR: sc-44250-PR (20 μ l, 434 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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