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CIS shRNA (h) Lentiviral Particles: sc-43685-V

BACKGROUND

Src homology (SH2) domains are noncatalytic sequences that are conserved among a number of cytoplasmic signaling proteins. These signaling proteins are directly regulated by receptor tyrosine kinases and control the activation of mitogenic signal transduction pathways by such receptors. For instance, ligand-induced activation of the EGF and PDGF receptors induces dimerization, triggers receptor autophosphorylation on tyrosine residues and results in the binding of a number of cytoplasmic SH2 domain proteins, such as PLC γ 1, Ras GAP and PI 3-kinase p85 to the activated receptors. The Shc gene encodes proteins with a single SH2 domain but no identifiable catalytic domain. CIS, cytokine-inducible SH2-containing protein, is a 267 amino acid protein with a single 96 amino acid SH2 domain that associates the tyrosine-phosphorylated β chain of the IL-3 receptor with the tyrosine-phosphorylated Epo receptor. CIS was initially described as an immediate early cytokine-responsive gene and appears to be a unique regulator of cytokine signaling.

REFERENCES

- Ullrich, A., et al. 1990. Signal transduction by receptors with tyrosine kinase activity. *Cell* 61: 203-212.
- Ellis, C., et al. 1990. Phosphorylation of GAP and GAP-associated proteins by transforming and mitogenic tyrosine kinases. *Nature* 343: 377-381.
- Morrison, D.K., et al. 1990. Platelet-derived growth factor (PDGF)-dependent association of phospholipase C- γ with the PDGF receptor signaling complex. *Mol. Cell. Biol.* 10: 2359-2366.
- Cantley, L.C., et al. 1991. Oncogenes and signal transduction. *Cell* 64: 281-302.
- McGlade, J., et al. 1992. Shc proteins are phosphorylated and regulated by the v-Src and v-Fps protein-tyrosine kinases. *Proc. Natl. Acad. Sci. USA* 89: 8869-8873.

CHROMOSOMAL LOCATION

Genetic locus: CISH (human) mapping to 3p21.2.

PRODUCT

CIS 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 lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CIS siRNA (h): sc-43685 and CIS shRNA Plasmid (h): sc-43685-SH as alternate gene silencing products.

APPLICATIONS

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

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.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 lentiviral transducing particles per milliliter; contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

CIS (H-80): sc-15344 is recommended as a control antibody for monitoring of CIS 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 CIS gene expression knockdown using RT-PCR Primer: CIS (h)-PR: sc-43685-PR (20 μ l, 431 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.

PROTOCOLS

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