

CIS siRNA (h): sc-43685

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. and Schlessinger, J. 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.
- Ravichandran, K.S., et al. 1993. Interaction of Shc with the ζ chain of the T cell receptor upon T cell activation. *Science* 262: 902-905.
- Yoshimura, A., et al. 1995. A novel cytokine-inducible gene CIS encodes an SH2-containing protein that binds to tyrosine-phosphorylated interleukin 3 and erythropoietin receptors. *EMBO J.* 14: 2816-2826.

CHROMOSOMAL LOCATION

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

PRODUCT

CIS siRNA (h) 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 CIS shRNA Plasmid (h): sc-43685-SH and CIS shRNA (h) Lentiviral Particles: sc-43685-V as alternate gene silencing products.

For independent verification of CIS (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43685A, sc-43685B and sc-43685C.

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

CIS siRNA (h) is recommended for the inhibition of CIS expression in human 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

CIS (D-8): sc-74581 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 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 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° 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.