SANTA CRUZ BIOTECHNOLOGY, INC.

Csk siRNA (m): sc-38971

BACKGROUND

All members of the Src gene family of tyrosine kinases are characterized by a carboxy terminal domain tyrosine which is highly phosphorylated in the inactive form of the enzyme and phosphorylated to a much lesser extent when the enzyme is active. In the case of Src p60, Y527 is this tyrosine; however, a mutant form of c-Src in which Y527 is replaced by phenylalanine is transforming and displays 5- to 10-fold elevated kinase activity compared to its normal counterpart. Csk has been identified as a Src-related tyrosine kinase having both SH2 and SH3 domains and a catalytic domain but lacking sequences amino terminal to the SH3 domain as well as carboxy terminal regulatory sequences. Csk phosphorylates Src on Y527 and also down-regulates Lyn, Fyn and Lck by tyrosine phosphorylation of carboxy terminal regulatory sites.

REFERENCES

1. Okada, M., et al. 1989. A protein tyrosine kinase involved in regulation of $pp60^{c\text{-}Src}$ function. J. Biol. Chem. 264: 20886-20893.
2. Nada, S., et al. 1991. Cloning of a complementary DNA for a protein-tyrosine kinase that specifically phosphorylates a negative regulatory site of $p60^{c\text{-}Src}$. Nature 351: 69-72.
3. Cooper, J.A., et al. 1993. The when and how of Src regulation. Cell 73: 1051-1054.
4. Bräuninger, A., et al. 1993. Characterization of the human Csk locus. Oncogene 8: 1365-1369.
5. Chow, L.M., et al. 1993. Negative regulation of T-cell receptor signalling by tyrosine protein kinase $p50^{Csk}$. Nature 365: 156-159.
6. Imamoto, A., et al. 1993. Disruption of the Csk gene, encoding a negative regulator of Src family tyrosine kinases, leads to neural tube defects and embryonic lethality in mice. Cell 73: 1117-1124.
7. Nada, S., et al. 1993. Constitutive activation of Src family kinases in mouse embryos that lack Csk. Cell 73: 1125-1135.
8. Superti-Furga, G., et al. 1993. Csk inhibition of c-Src activity requires both the SH2 and SH3 domains of Src. EMBO J. 12: 2625-2634.

CHROMOSOMAL LOCATION

Genetic locus: Csk (mouse) mapping to 9 B.

PRODUCT

Csk siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Csk shRNA Plasmid (m): sc-38971-SH and Csk shRNA (m) Lentiviral Particles: sc-38971-V as alternate gene silencing products.

For independent verification of Csk (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-38971A, sc-38971B and sc-38971C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Csk siRNA (m) is recommended for the inhibition of Csk expression in mouse 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 µM in 66 µl. 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

Csk (E-3): sc-166560 is recommended as a control antibody for monitoring of Csk 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Csk gene expression knockdown using RT-PCR Primer: Csk (m)-PR: sc-38971-PR (20 µl). 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.