SANTA CRUZ BIOTECHNOLOGY, INC.

Sin siRNA (h): sc-40796

BACKGROUND

A protein designated p130 Cas (for Crk-associated substrate), represents one of several known substrates for v-Crk encoded p47. p130 Cas (also designated breast cancer anti-estrogen resistance protein 1 or Cas scaffolding protein family member 1), exhibits a high level of tyrosine phosphorylation and is tightly associated with v-Crk, suggesting a role in v-Crk-mediated cell signaling. p130 Cas is a novel SH3-containing signaling molecule with a cluster of multiple putative SH2-binding motifs for v-Crk. Two p130 Cas related proteins, designated Sin (Src interacting or signal integrating protein, also designated Cas3 or HEFS) and Cas-L (human enhancer of filamentatin 1, HEF1 or Cas3), have also been identified. Sin contains SH2/SH3 domains and has been shown to activate Src. Cas-L contains an SH3 domain and has been shown to be a docking protein that serves as a substrate for phosphorylation by several oncogenic tyrosine kinases.

REFERENCES

1. Kanner, S.B., et al. 1991. The SH2 and SH3 domains of pp60src direct stable association with tyrosine phosphorylated proteins p130 and p110. EMBO J. 10: 1689-1698.
2. Matusda, M., et al. 1991. Identification of domain of the v-Crk oncogene product sufficient for association with phosphotyrosine-containing proteins. Mol. Cell. Biol. 11: 1607-1613.
3. Birge, R.B., et al. 1992. Tyrosine-phosphorylated epidermal growth factor receptor and cellular p130 provide high-affinity binding substrates to analyze Crk-phosphotyrosine-dependent interactions *in vitro*. J. Biol. Chem. 267: 10588-10595.
4. Matsuda, M., et al. 1992. Two species of human Crk cDNA encode proteins with distinct biological activities. Mol. Cell. Biol. 12: 3482-3489.
5. Sakai, R., et al. 1994. A novel signaling molecule, p130, forms stable complexes *in vivo* with v-Crk and v-Src in a tyrosine phosphorylation-dependent manner. EMBO J. 13: 3748-3756.
6. Alexandropoulos, K. and Baltimore, D. 1996. Coordinate activation of c-Src by SH3- and SH2-binding sites on a novel p130 Cas-related protein, Sin. Genes Dev. 10: 1341-1355.

CHROMOSOMAL LOCATION

Genetic locus: EFS (human) mapping to 14q11.2.

PRODUCT

Sin 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Sin shRNA Plasmid (h): sc-40796-SH and Sin shRNA (h) Lentiviral Particles: sc-40796-V as alternate gene silencing products.

For independent verification of Sin (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-40796A, sc-40796B and sc-40796C.

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

Sin siRNA (h) is recommended for the inhibition of Sin 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 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

Sin (13): sc-136329 is recommended as a control antibody for monitoring of Sin 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 Sin gene expression knockdown using RT-PCR Primer: Sin (h)-PR: sc-40796-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.