SANTA CRUZ BIOTECHNOLOGY, INC.

Crk-L siRNA (m): sc-35115

BACKGROUND

SH2 and SH3 (Src homology) domains were originally identified as critical functional domains within non-receptor proteins with tyrosine kinase activity. One of the first members of the family to be identified, Crk, is a transformation-specific protein that induces elevation of cellular phosphotyrosine levels, but lacks tyrosine kinase activity itself. A second protein, Nck, consists solely of three SH3 domains and one SH2 domain, while GRB2 contains an SH2 domain flanked on both sides by SH3 domains. A member of this protein class, Crk-L, is encoded by a gene located on chromosome 22q11.21, band 11, centromeric of the chronic myelogenous leukemia breakpoint region. Crk-L encodes a 303 amino acid protein with one SH2 and two SH3 domains.

REFERENCES

1. Mayer, B., et al. 1988. A novel viral oncogene with structural similarity to phospholipase C. Nature 332: 272-275.
2. Lehmann, J.M., et al. 1990. Nck, a melanoma cDNA encoding a cytoplasmic protein consisting of the Src homology units SH2 and SH3. Nucleic Acids Res. 18: 1048.
3. Koch, C.A., et al. 1991. SH2 and SH3 domains: elements that control interactions of cytoplasmic signaling proteins. Science 252: 668-674.
4. Park, D., et al. 1992. Phosphorylation of Nck in response to a variety of receptors, phorbol myristate acetate, and cyclic AMP. Mol. Cell. Biol. 12: 5816-5823.
5. Lowenstein, et al. 1992. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. Cell 70: 431-442.
6. Pawson, T., et al. 1992. SH2 and SH3 domains: from structure to function. Cell 71: 359-362.
7. Williams, L.T. 1992. Missing links between receptors and Ras. Curr. Biol. 2: 601-603.
8. ten Hoeve, J., et al. 1993. Isolation and chromosomal localization of Crk-L, a human Crk-like gene. Oncogene 8: 2469-2474.

CHROMOSOMAL LOCATION

Genetic locus: Crkl (mouse) mapping to 16 A3.

PRODUCT

Crk-L 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 Crk-L shRNA Plasmid (m): sc-35115-SH and Crk-L shRNA (m) Lentiviral Particles: sc-35115-V as alternate gene silencing products.

For independent verification of Crk-L (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-35115A, sc-35115B and sc-35115C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Crk-L shRNA Plasmid (m) is recommended for the inhibition of Crk-L 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

Crk-L (B-1): sc-365092 is recommended as a control antibody for monitoring of Crk-L 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 Crk-L gene expression knockdown using RT-PCR Primer: Crk-L (m)-PR: sc-35115-PR (20 µl, 464 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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