



Crk-L siRNA (h): sc-35114

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. Lowenstein, E.J., et al. 1992. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to Ras signaling. *Cell* 70: 431-442.

CHROMOSOMAL LOCATION

Genetic locus: CRKL (human) mapping to 22q11.21.

PRODUCT

Crk-L 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 Crk-L shRNA Plasmid (h): sc-35114-SH and Crk-L shRNA (h) Lentiviral Particles: sc-35114-V as alternate gene silencing products.

For independent verification of Crk-L (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-35114A, sc-35114B and sc-35114C.

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

Crk-L siRNA (h) is recommended for the inhibition of Crk-L 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

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-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 Crk-L gene expression knockdown using RT-PCR Primer: Crk-L (h)-PR: sc-35114-PR (20 μ l, 464 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Mintz, P.J., et al. 2009. An unrecognized extracellular function for an intracellular adapter protein released from the cytoplasm into the tumor microenvironment. *Proc. Natl. Acad. Sci. USA* 106: 2182-2187.
2. Waight, J.D., et al. 2014. Regulation of the interferon regulatory factor-8 (IRF-8) tumor suppressor gene by the signal transducer and activator of transcription 5 (Stat5) transcription factor in chronic myeloid leukemia. *J. Biol. Chem.* 289: 15642-15652.
3. Hrnicius, E.R., et al. 2014. Avian influenza viruses inhibit the major cellular signalling integrator c-Abl. *Cell. Microbiol.* 16: 1854-1874.
4. Cheng, S., et al. 2015. Crk-like adapter protein regulates CCL19/CCR7-mediated epithelial-to-mesenchymal transition via ERK signaling pathway in epithelial ovarian carcinomas. *Med. Oncol.* 32: 47.
5. Han, G., et al. 2015. Crk-L mediates CCL20/CCR6-induced EMT in gastric cancer. *Cytokine* 76: 163-169.

RESEARCH USE

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