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Crk II siRNA (m): sc-44854

BACKGROUND

The Crk family of adapter proteins including Crk-II, Crk-I, and Crk-L consist mostly of SH2 and SH3 domains. Through the interactions between SH2 domain and phosphotyrosine residues and/or between SH3 domain and proline-rich motifs, they are involved in a variety of signaling cascades. Crk I and Crk II are encoded by the same gene, which undergoes alternative splicing to yield these two proteins, but differ in their biological activities. Crk-II has less transforming activity than Crk-I, although both Crk-I and Crk-II bind to many tyrosine-phosphorylated proteins that bind to grb2. In addition, Crk-II becomes rapidly tyrosine-phosphorylated in response to stimulation with Insulin-like growth factor I (IGF-I) and might be involved in the IGF-I receptor signalling pathway. The gene encoding Crk I and II maps to human chromosome 17p13, a region which demonstrates frequent deletion or loss of heterozygosity in a wide range of human cancers.

REFERENCES

1. Mayer, B.J., et al. 1990. Association of the v-Crk oncogene product with phosphotyrosine-containing proteins and protein kinase activity. *Proc. Natl. Acad. Sci. USA* 87: 2638-2642.
2. Matsuda, M., et al. 1990. Binding of transforming protein, P47gag-Crk, to a broad range of phosphotyrosine-containing proteins. *Science* 248: 1537-1539.
3. Mayer, B.J., et al. 1990. Mutagenic analysis of the v-Crk oncogene: requirement for SH2 and SH3 domains, and correlation between increased cellular phosphotyrosine and transformation. *J. Virol.* 64: 3581-3589.
4. Matsuda, M., et al. 1992. Two species of human CRK cDNA encode proteins with distinct biological activities. *Mol. Cell. Biol.* 12: 3482-3489.

CHROMOSOMAL LOCATION

Genetic locus: Crk (mouse) mapping to 11 B5.

PRODUCT

Crk II 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Crk II shRNA Plasmid (m): sc-44854-SH and Crk II shRNA (m) Lentiviral Particles: sc-44854-V as alternate gene silencing products.

For independent verification of Crk II (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-44854A, sc-44854B and sc-44854C.

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 II siRNA (m) is recommended for the inhibition of Crk II 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 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 II (B-4): sc-390132 is recommended as a control antibody for monitoring of Crk II 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 MarkerTM 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 II gene expression knockdown using RT-PCR Primer: Crk II (m)-PR: sc-44854-PR (20 μ l, 557 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.