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Crk I/II shRNA (h) Lentiviral Particles: sc-43704-V

BACKGROUND

The Crk family of adapter proteins, including Crk I, Crk II 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. Matsuda, M., et al. 1992. Two species of human CRK cDNA encode proteins with distinct biological activities. *Mol. Cell. Biol.* 12: 3482-3489.
2. Fioretos, T., et al. 1993. CRK proto-oncogene maps to human chromosome band 17p13. *Oncogene* 10: 2853-2855.
3. Koval, A.P., et al. 1998. Interaction *in vitro* of the product of the c-Crk II proto-oncogene with the Insulin-like growth factor I receptor. *Biochem. J.* 330: 923-932
4. Imaizumi, T., et al. 1999. Mutant mice lacking Crk II caused by the gene trap insertional mutagenesis: Crk II is not essential for embryonic development. *Biochem. Biophys. Res. Commun.* 266: 569-574.
5. SWISS-PROT/TrEMBL. (1169096) World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: CRK (human) mapping to 17p13.3.

PRODUCT

Crk I/II shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Crk I/II siRNA (h): sc-43704 and Crk I/II shRNA Plasmid (h): sc-43704-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

Crk I/II shRNA (h) Lentiviral Particles is recommended for the inhibition of Crk I expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Crk I/II (D-6): sc-393160 is recommended as a control antibody for monitoring of Crk I/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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Crk I gene expression knockdown using RT-PCR Primer: Crk I/II (h)-PR: sc-43704-PR (20 μ l, 425 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.