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DOCK 180 shRNA (h2) Lentiviral Particles: sc-44294-V

BACKGROUND

The v-Crk oncogene product shares homologous amino acid sequences, designated Src homology region 2 (SH2) and SH3, with many molecules involved in signal transduction. The v-Crk cellular homolog, c-Crk, is a member of a newly emerging class of genes including Nck and GRB2/ASH which encode proteins that consist primarily of SH2 and SH3 domains. Two distinct human c-Crk cDNAs, designated Crk I and Crk II, have been identified and shown to represent alternative splice products of c-Crk. The major translational product of c-Crk I has been identified as a variably expressed protein, while c-Crk II encodes a widely expressed protein and a more variably expressed protein. The major c-Crk transforming activity appears associated with c-Crk I p28 expression. DOCK 180, a protein downstream of Crk, has been identified as a major Crk-associated protein. When DOCK 180 is recruited to the plasma membrane from a cytoplasmic reservoir, presumably by Crk, changes in cellular morphology and spindle formation occur, suggesting DOCK 180 to be a Crk effector molecule.

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.
5. Matsuda, M., et al. 1992. Biological and biochemical activity of v-Crk chimeras containing the SH2/SH3 regions of phosphatidylinositol-specific phospholipase C- γ and Src. *J. Virol.* 66: 115-121.

CHROMOSOMAL LOCATION

Genetic locus: DOCK1 (human) mapping to 10q26.2.

PRODUCT

DOCK 180 shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 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 DOCK 180 siRNA (h2): sc-44294 and DOCK 180 shRNA Plasmid (h2): sc-44294-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.

APPLICATIONS

DOCK 180 shRNA (h2) Lentiviral Particles is recommended for the inhibition of DOCK 180 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

DOCK 180 (E-2): sc-514080 is recommended as a control antibody for monitoring of DOCK 180 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 DOCK 180 gene expression knockdown using RT-PCR Primer: DOCK 180 (h2)-PR: sc-44294-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

PROTOCOLS

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