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SANTA CRUZ BIOTECHNOLOGY, INC.

Dok-3 shRNA (h) Lentiviral Particles: sc-44762-V

BACKGROUND

Dok-1, Dok-2 and Dok-3 are members of a class of "docking" proteins which contain multiple tyrosine residues and putative SH2 binding sites. Dok-1 associates with the Ras GTPase activating protein (Ras GAP) upon tyrosine phosphorylation. Dok-2 (also designated p56 Dok) has also been identified as a potential mediator of the effects of p210 Bcr-Abl. Dok-3 is an adapter involved in the recruitment of inhibitory molecules and is highly expressed in B cells and macrophages. Immunoreceptor-mediated cellular activation induces tyrosine phosphorylation of Dok-3. Upon phosphorylation, Dok-3 binds to 5' inositol phosphatase SHIP and the protein tyrosine kinase Csk. Dok-3 may play a significant role in the negative regulation of immunoreceptor signaling in hemopoietic cells.

REFERENCES

1. Wisniewski, D., et al. 1994. A 62-kilodalton tyrosine phosphoprotein constitutively present in primary chronic phase chronic myelogenous leukemia enriched lineage negative blast populations. Leukemia 8: 688-693.
2. Mayer, B.J., et al. 1995. Evidence that SH2 domains promote processive phosphorylation by protein-tyrosine kinases. Curr. Biol. 5: 296-305.
3. Carpino, N., et al. 1997. p62dok: a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells. Cell 88: 197-204.
4. Yamanashi, Y., et al. 1997. Identification of the Abl- and rasGAP-associated 62 kDa protein as a docking protein, Dok. Cell 88: 205-211.
5. Di Cristofano, A., et al. 1998. Molecular cloning and characterization of p56dok-2 defines a new family of RasGAP-binding proteins. J. Biol. Chem. 273: 4827-4830.
6. Cong, F., et al. 1999. Characterization of a novel member of the DOK family that binds and modulates Abl signaling. Mol. Cell. Biol. 19: 8314-8325.

CHROMOSOMAL LOCATION

Genetic locus: DOK3 (human) mapping to 5q35.3.

PRODUCT

Dok-3 shRNA (h) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 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 Dok-3 siRNA (h): sc-44762 and Dok-3 shRNA Plasmid (h): sc-44762-SH as alternate gene silencing products.

APPLICATIONS

Dok-3 shRNA (h) Lentiviral Particles is recommended for the inhibition of Dok-3 expression in human cells.

PROTOCOLS

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 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

Dok-3 (F-7): sc-390007 is recommended as a control antibody for monitoring of Dok-3 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 Dok-3 gene expression knockdown using RT-PCR Primer: Dok-3 (h)-PR: sc-44762-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

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.