



Dok-3 siRNA (m): sc-35213

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. and Baltimore, D. 1997. Identification of the Abl- and Ras GAP-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 Ras GAP-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 (mouse) mapping to 13 B1.

PRODUCT

Dok-3 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 Dok-3 shRNA Plasmid (m): sc-35213-SH and Dok-3 shRNA (m) Lentiviral Particles: sc-35213-V as alternate gene silencing products.

For independent verification of Dok-3 (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-35213A, sc-35213B and sc-35213C.

PROTOCOLS

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

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

Dok-3 siRNA (m) is recommended for the inhibition of Dok-3 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

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 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 Dok-3 gene expression knockdown using RT-PCR Primer: Dok-3 (m)-PR: sc-35213-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

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