

Dok-1 siRNA (r): sc-270314

BACKGROUND

Dok-1 associates with the Ras GTPase-activating protein (Ras GAP) upon tyrosine phosphorylation. Evidence suggests that Dok-1 (also designated p62^{dok}) is a substrate of the constitutive tyrosine kinase activity of p210 Bcr-Abl, a fusion protein caused by the t(9;22) translocation and associated with chronic myelogenous leukemia. Dok-1, as well as the tyrosine kinase substrates IRS-1 and Cas, are members of a class of "docking" proteins which contain multiple tyrosine residues and putative SH2 binding sites. Dok-1 is suspected to be the substrate phosphorylated in response to stimulation by a number of growth factors, including PDGF, VEGF, Insulin and IGF. Dok-2 (also designated p56^{dok}) has also been identified as a potential mediator of the effects of p210 Bcr-Abl.

REFERENCES

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2. Myers, M.G., et al. 1994. The IRS-1 signaling system. *Trends Biochem. Sci.* 19: 289-293.
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4. Mayer, B.J., et al. 1995. Evidence that SH2 domains promote processive phosphorylation by protein-tyrosine kinases. *Curr. Biol.* 5: 296-305.
5. Holgado, M.M., et al. 1996. A Grb2-associated docking protein in EGF and Insulin receptor signalling. *Nature* 379: 560-564.
6. Carpino, N., et al. 1997. p62^{dok}: a constitutively tyrosine-phosphorylated, GAP-associated protein in chronic myelogenous leukemia progenitor cells. *Cell* 88: 197-204.
7. Yamanashi, Y., et al. 1997. Identification of the Abl- and Ras GAP-associated 62 kDa protein as a docking protein, Dok. *Cell* 88: 205-211.
8. Di Cristofano, A., et al. 1998. Molecular cloning and characterization of p56^{dok-2} defines a new family of Ras GAP-binding proteins. *J. Biol. Chem.* 273: 4827-4830.

CHROMOSOMAL LOCATION

Genetic locus: Dok1 (rat) mapping to 4q34.

PRODUCT

Dok-1 siRNA (r) 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-1 shRNA Plasmid (r): sc-270314-SH and Dok-1 shRNA (r) Lentiviral Particles: sc-270314-V as alternate gene silencing products.

For independent verification of Dok-1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270314A, sc-270314B and sc-270314C.

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-1 siRNA (r) is recommended for the inhibition of Dok-1 expression in rat 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-1 (A-3): sc-6929 is recommended as a control antibody for monitoring of Dok-1 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-1 gene expression knockdown using RT-PCR Primer: Dok-1 (r)-PR: sc-270314-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.

PROTOCOLS

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