



# Cot siRNA (m): sc-35096

## BACKGROUND

The role of mitogen-activated protein kinases (MAPKs) in cell signaling pathways is well established. The rat gene *Tpl-2*, for tumor progression locus 2, and the human and mouse homologues *c-Cot*, for cancer Osaka thyroid oncogene, encode a proto-oncogene serine/threonine protein kinase that was shown to play a role in the functional activation of the MAP kinase pathway. Overexpression of Cot induces MAP kinase activation in COS-1 and NIH/3T3 cells. Cot-mediated activation of MAP kinase is inhibited by both Ras N17, a dominant negative mutant of c-H-Ras, and Raf-1s621A, a dominant negative mutant of Raf-1, suggesting that Cot functions upstream of Ras and Raf-1. Other studies have shown that a kinase-negative, dominant negative mutant of Cot partially blocks Ras or Raf-1-induced MAP kinase activation, arguing that Cot functions downstream of Ras and Raf-1. To explain these contrasting findings, it has been suggested that Cot, Ras and Raf-1 may form a multimeric complex that phosphorylates MEK-1. Cot has also been shown to be implicated in T lymphocyte activation. Two forms of Cot are produced by alternative initiation of translation.

## REFERENCES

1. Haubruk, H., et al. 1991. Ras p21: effects and regulation. *Biochem. Biophys. Acta* 1072: 215-229.
2. Roberts, T.M. 1992. A signal chain of events. *Nature* 360: 534-535.
3. Nishida, E., et al. 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. *Trends Biochem. Sci.* 18: 128-131.
4. Fabian, J.R., et al. 1993. Requirement for Raf and MAP kinase function during the meiotic maturation of *Xenopus* oocytes. *J. Cell Biol.* 122: 645-652.
5. Aoki, M., et al. 1993. The human Cot proto-oncogene encodes two protein serine/threonine kinases with different transforming activities by alternative initiation of translation. *J. Biol. Chem.* 268: 22723-22732.
6. Patriotis, C., et al. 1994. *Tpl-2* acts in concert with Ras and Raf-1 to activate mitogen-activated protein kinase. *Proc. Natl. Acad. Sci. USA* 91: 9755-9759.

## CHROMOSOMAL LOCATION

Genetic locus: Map3k8 (mouse) mapping to 18 A1.

## PRODUCT

Cot 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cot shRNA Plasmid (m): sc-35096-SH and Cot shRNA (m) Lentiviral Particles: sc-35096-V as alternate gene silencing products.

For independent verification of Cot (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-35096A, sc-35096B and sc-35096C.

## RESEARCH USE

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

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

Cot shRNA (m) Lentiviral Particles is recommended for the inhibition of Cot 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  $\mu$ M in 66  $\mu$ 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

Cot (H-7): sc-373677 is recommended as a control antibody for monitoring of Cot 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 Cot gene expression knockdown using RT-PCR Primer: Cot (m)-PR: sc-35096-PR (20  $\mu$ l, 599 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.