SANTA CRUZ BIOTECHNOLOGY, INC.

Cot siRNA (h): sc-35095

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. Cell biology. A signal chain of events. Nature 360: 534-535.

CHROMOSOMAL LOCATION

Genetic locus: MAP3K8 (human) mapping to 10p11.23.

PRODUCT

Cot siRNA (h) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Cot shRNA Plasmid (h): sc-35095-SH and Cot shRNA (h) Lentiviral Particles: sc-35095-V as alternate gene silencing products.

For independent verification of Cot (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35095A, sc-35095B and sc-35095C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Cot siRNA (h) is recommended for the inhibition of Cot expression in human 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 µM in 66 µl. 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 (h)-PR: sc-35095-PR (20 µl, 425 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Cismasiu, V.B., et al. 2009. BCL11B enhances TCR/CD28-triggered NFκB activation through up-regulation of Cot kinase gene expression in T-lymphocytes. Biochem. J. 417: 457-466.
2. Martel, G., et al. 2013. The protein kinase TPL2 is essential for ERK1/ERK2 activation and cytokine gene expression in airway epithelial cells exposed to pathogen-associated molecular patterns (PAMPs). PLoS ONE 8: e59116.
3. Schmid, S., et al. 2014. Mitogen-activated protein kinase-mediated licensing of interferon regulatory factor 3/7 reinforces the cell response to virus. J. Biol. Chem. 289: 299-311.
4. Sheu, W.H., et al. 2021. Therapeutic potential of Tpl2 (tumor progression locus 2) inhibition on diabetic vasculopathy through the blockage of the inflammasome complex. Arterioscler. Thromb. Vasc. Biol. 41: e46-e62.

RESEARCH USE

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