

CNOT10 siRNA (h): sc-78124

BACKGROUND

CNOT10 is a subunit of the CCR4-NOT complex which consists of at least five other CNOT subunit proteins and TAB182. The CCR4-NOT complex is an evolutionarily conserved, multi-component complex known to be involved in transcription, as well as in mRNA degradation. Various subunits (e.g. CNOT1, CNOT3) are uniquely involved in influencing nuclear hormone receptor activities. In effect, this complex has an important role as a transcription regulator and repressor of nuclear receptor signaling that is relevant to the molecular pathways involved in cancer. The CCR4-NOT complex is also involved in the regulation of Histone H3 lysine 4 methylation through a ubiquitin-dependent pathway that likely involves the proteasome.

REFERENCES

1. Mulder, K.W., et al. 2005. DNA damage and replication stress induced transcription of RNR genes is dependent on the Ccr4-Not complex. *Nucleic Acids Res.* 33: 6384-6392.
2. Winkler, G.S., et al. 2006. Human Ccr4-Not complex is a ligand-dependent repressor of nuclear receptor-mediated transcription. *EMBO J.* 25: 3089-3099.
3. Behm-Ansmant, I., et al. 2006. mRNA degradation by miRNAs and GW182 requires both CCR4:NOT deadenylase and DCP1:DCP2 decapping complexes. *Genes Dev.* 20: 1885-1898.
4. Lenssen, E., et al. 2007. The Ccr4-Not complex regulates Skn7 through Srb10 kinase. *Eukaryot. Cell* 6: 2251-2259.
5. James, N., et al. 2007. A SAGA-independent function of SPT3 mediates transcriptional deregulation in a mutant of the Ccr4-Not complex in *Saccharomyces cerevisiae*. *Genetics* 177: 123-135.
6. Wagner, E., et al. 2007. An unconventional human Ccr4-Caf1 deadenylase complex in nuclear cajal bodies. *Mol. Cell. Biol.* 27: 1686-1695.

CHROMOSOMAL LOCATION

Genetic locus: CNOT10 (human) mapping to 3p22.3.

PRODUCT

CNOT10 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CNOT10 shRNA Plasmid (h): sc-78124-SH and CNOT10 shRNA (h) Lentiviral Particles: sc-78124-V as alternate gene silencing products.

For independent verification of CNOT10 (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-78124A, sc-78124B and sc-78124C.

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

CNOT10 siRNA (h) is recommended for the inhibition of CNOT10 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 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

CNOT10 (H-9): sc-514570 is recommended as a control antibody for monitoring of CNOT10 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 CNOT10 gene expression knockdown using RT-PCR Primer: CNOT10 (h)-PR: sc-78124-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.