

CNOT4 siRNA (m): sc-72942

BACKGROUND

The CCR4-NOT complex is an evolutionarily conserved, multi-component complex known to be involved in transcription as well as mRNA degradation. Various subunits within the complex are involved in influencing nuclear hormone receptor activities. 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. CNOT4 (CCR4-NOT transcription complex subunit 4), also known as CCR4-associated factor 4 and E3 ubiquitin-protein ligase CNOT4, is a 575 amino acid protein that is a subunit of the CCR4-NOT complex. CNOT4 contains one C3H1-type zinc finger, one RING-type zinc finger and one RRM (RNA recognition motif) domain. Via its RING domain, CNOT4 binds E2 ubiquitin ligases. CNOT4 functions as a UbcH5B-dependent ubiquitin-protein ligase (E3 ligase). There are eight isoforms of CNOT4 that are expressed as a result of alternative splicing events.

REFERENCES

1. Albert, T.K., et al. 2000. Isolation and characterization of human orthologs of yeast CCR4-NOT complex subunits. *Nucleic Acids Res.* 28: 809-817.
2. Hanzawa, H., et al. 2001. The structure of the C4C4 ring finger of human NOT4 reveals features distinct from those of C3HC4 RING fingers. *J. Biol. Chem.* 276: 10185-10190.
3. Albert, T.K., et al. 2002. Identification of a ubiquitin-protein ligase subunit within the CCR4-NOT transcription repressor complex. *EMBO J.* 21: 355-364.
4. Dominguez, C., et al. 2004. Structural model of the UbcH5B/CNOT4 complex revealed by combining NMR, mutagenesis, and docking approaches. *Structure* 12: 633-644.
5. Online Mendelian Inheritance in Man, OMIM[™]. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 604911. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: Cnot4 (mouse) mapping to 6 B1.

PRODUCT

CNOT4 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 CNOT4 shRNA Plasmid (m): sc-72942-SH and CNOT4 shRNA (m) Lentiviral Particles: sc-72942-V as alternate gene silencing products.

For independent verification of CNOT4 (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-72942A, sc-72942B and sc-72942C.

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

CNOT4 siRNA (m) is recommended for the inhibition of CNOT4 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

CNOT4 (343CT14.6.5): sc-517324 is recommended as a control antibody for monitoring of CNOT4 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 CNOT4 gene expression knockdown using RT-PCR Primer: CNOT4 (m)-PR: sc-72942-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.