

CNOT8 siRNA (h): sc-72948

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. CNOT8 (CCR4-NOT transcription complex subunit 8), also known as CALIF or POP2, is a 292 amino acid protein that localizes to both the nucleus and the cytoplasm and functions as part of the CCR-NOT complex. Expressed ubiquitously, CNOT8 plays a role in transcriptional regulation for a diverse set of processes.

REFERENCES

1. Bogdan, J.A., et al. 1998. Human carbon catabolite repressor protein (CCR4)-associative factor 1: cloning, expression and characterization of its interaction with the B-cell translocation protein BTG1. *Biochem. J.* 336: 471-481.
2. Fidler, C., et al. 1999. The human POP2 gene: identification, sequencing, and mapping to the critical region of the 5q-syndrome. *Genomics* 56: 134-136.
3. Albert, T.K., et al. 2000. Isolation and characterization of human orthologs of yeast CCR4-NOT complex subunits. *Nucleic Acids Res.* 28: 809-817.
4. Prévôt, D., et al. 2001. Relationships of the antiproliferative proteins BTG1 and BTG2 with CAF1, the human homolog of a component of the yeast CCR4 transcriptional complex: involvement in estrogen receptor α signaling pathway. *J. Biol. Chem.* 276: 9640-9648.
5. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 603731. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
6. Morel, A.P., et al. 2003. BTG2 antiproliferative protein interacts with the human CCR4 complex existing *in vivo* in three cell-cycle-regulated forms. *J. Cell Sci.* 116: 2929-2936.

CHROMOSOMAL LOCATION

Genetic locus: CNOT8 (human) mapping to 5q33.2.

PRODUCT

CNOT8 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 CNOT8 shRNA Plasmid (h): sc-72948-SH and CNOT8 shRNA (h) Lentiviral Particles: sc-72948-V as alternate gene silencing products.

For independent verification of CNOT8 (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-72948A, sc-72948B and sc-72948C.

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 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

CNOT8 siRNA (h) is recommended for the inhibition of CNOT8 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

CNOT8 (1F11): sc-293395 is recommended as a control antibody for monitoring of CNOT8 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 CNOT8 gene expression knockdown using RT-PCR Primer: CNOT8 (h)-PR: sc-72948-PR (20 μ l, 434 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 for detailed protocols and support products.