SANTA CRUZ BIOTECHNOLOGY, INC.

CHCHD4 siRNA (h): sc-78156

BACKGROUND

CHCHD4 (coiled-coil-helix-coiled-coil-helix domain containing 4), also known as MIA40, is a 142 amino acid protein that contains one CHCH domain and localizes to the mitochondrial intermembrane space. Expressed in a variety of tissues with particularly high expression in kidney and liver, CHCHD4 exists as a homooligomer that is required for the folding of small cysteine-containing proteins. Specifically, CHCHD4 is thought to function via a disulfide relay system that oxidizes precursor proteins, thereby promoting their folding within mitochondria. CHCHD4 is expressed as multiple alternatively spliced isoforms that are encoded by a gene which maps to human chromosome 3. Chromosome 3 is made up of about 214 million bases encoding over 1,100 genes, including a chemokine receptor (CKR) gene cluster and a variety of human cancer-related gene loci.

REFERENCES

1. Chacinska, A., et al. 2004. Essential role of Mia40 in import and assembly of mitochondrial intermembrane space proteins. EMBO J. 23: 3735-3746.
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3. Terziyska, N., et al. 2007. The sulfhydryl oxidase Erv1 is a substrate of the Mia40-dependent protein translocation pathway. FEBS Lett. 581: 1098-1102.
4. Online Mendelian Inheritance in Man, OMIM™. 2007. Johns Hopkins University, Baltimore, MD. MIM Number: 611077. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Bihlmaier, K., et al. 2008. The disulfide relay of the intermembrane space of mitochondria: an oxygen-sensing system? Ann. N.Y. Acad. Sci. 1147: 293-302.
6. Chacinska, A., et al. 2008. Mitochondrial biogenesis, switching the sorting pathway of the intermembrane space receptor Mia40. J. Biol. Chem. 283: 29723-29729.
7. Koehler, C.M., et al. 2009. Redox regulation of protein folding in the mitochondrial intermembrane space. Biochim. Biophys. Acta 1793: 139-145.

CHROMOSOMAL LOCATION

Genetic locus: CHCHD4 (human) mapping to 3p25.1.

PRODUCT

CHCHD4 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 CHCHD4 shRNA Plasmid (h): sc-78156-SH and CHCHD4 shRNA (h) Lentiviral Particles: sc-78156-V as alternate gene silencing products.

For independent verification of CHCHD4 (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-78156A, sc-78156B and sc-78156C.

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

CHCHD4 siRNA (h) is recommended for the inhibition of CHCHD4 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

CHCHD4 (C-12): sc-365137 is recommended as a control antibody for monitoring of CHCHD4 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 CHCHD4 gene expression knockdown using RT-PCR Primer: CHCHD4 (h)-PR: sc-78156-PR (20 µl). 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.