

CCS siRNA (m): sc-29957

BACKGROUND

Cu-Zn superoxide dismutase-1 (SOD-1) is a well characterized cytosolic scavenger of oxygen free radicals that requires copper and zinc binding to potentiate its enzymatic activity. Copper chaperone for SOD-1 (CCS) is essential for the incorporation of copper into SOD-1, and therefore is necessary for its enzymatic activity. CCS prevents copper ions from binding to intracellular copper scavengers and provides the SOD-1 enzyme with the necessary copper cofactor. CCS escorts copper only to SOD-1 and fails to deliver copper to proteins in the mitochondria, nucleus or secretory pathway. CCS interacts with both wildtype and mutated forms of SOD-1 through CCS domains that are homologous in SOD-1. CCS exists as a homodimer that may form a heterodimer with SOD-1 during copper loading. While many tissues express CCS, the chaperone is most abundant in the kidney, liver and Purkinje cells in the neurophil of the central nervous system.

REFERENCES

1. Levanon, D., et al. 1985. Architecture and anatomy of the chromosomal locus in human chromosome 21 encoding the Cu-Zn superoxide dismutase. *EMBO J.* 4: 77-84.
2. Bewley, G.C. 1988. cDNA and deduced amino acid sequence of murine Cu-Zn superoxide dismutase. *Nucleic Acids Res.* 16: 2728.
3. Culotta, V.C., et al. 1997. The copper chaperone for superoxide dismutase. *J. Biol. Chem.* 272: 23469-23472.
4. Casareno, R.L., et al. 1998. The copper chaperone CSS directly interacts with Cu-Zn superoxide dismutase. *J. Biol. Chem.* 272: 23625-23628.
5. Rothstein, J.D., et al. 1999. The copper chaperone CCS is abundant in neurons and astrocytes in human and rodent brain. *J. Neurochem.* 72: 422-429.

CHROMOSOMAL LOCATION

Genetic locus: Ccs (mouse) mapping to 19 A.

PRODUCT

CCS 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 CCS shRNA Plasmid (m): sc-29957-SH and CCS shRNA (m) Lentiviral Particles: sc-29957-V as alternate gene silencing products.

For independent verification of CCS (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-29957A, sc-29957B and sc-29957C.

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.

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

CCS shRNA (m) Lentiviral Particles is recommended for the inhibition of CCS 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

CCS (H-7): sc-55561 is recommended as a control antibody for monitoring of CCS 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 CCS gene expression knockdown using RT-PCR Primer: CCS (m)-PR: sc-29957-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.