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CSP siRNA (h): sc-43709

BACKGROUND

Cysteine string proteins (CSPs) are synaptic vesicle-associated, secretory vesicle proteins that are involved in Ca^{2+} -regulated exocytosis of synaptic vesicles and modulation of presynaptic transmembrane calcium fluxes in neuroendocrine and endocrine cell types. CSP contains a J-domain that binds HSP 70/HSC 70 chaperone ATPases and a membrane-targeting, palmitoylated cysteine-rich string region. CSPs may act as molecular chaperones in synapses, and mediate conformational folding of components of the vesicular exocytotic machinery. CSP is involved in the fine tuning of neurotransmission through its interaction with receptor-coupled trimeric GTP binding proteins (G proteins) and N-type Ca^{2+} channels. Two variants of CSP have been described: CSP1; and the 31 amino acid, C-terminally truncated isoform, CSP2. Subcellular fractionation of insulinoma cells shows CSP1 in granular fractions, while the membrane and cytosol fractions contain predominantly CSP2. The fractions also contain additional proteins, presumably CSP dimers. Furthermore, in various mammalian cell lines (including rat brain) CSP1 expression predominates CSP2 expression.

REFERENCES

1. Brown, H., et al. 1998. Cysteine string protein (CSP) is an Insulin secretory granule-associated protein regulating β -cell exocytosis. *EMBO J.* 17: 5048-5058.
2. Chamberlain, L.H., et al. 1998. Cysteine string protein functions directly in regulated exocytosis. *Mol. Biol. Cell* 9: 2259-2267.
3. Zhang, H., et al. 1999. Mutational analysis of cysteine-string protein function in Insulin exocytosis. *J. Cell Sci.* 112: 1345-1351.
4. Magga, J.M., et al. 2000. Cysteine string protein regulates G protein modulation of N-type calcium channels. *Neuron* 28: 195-204.
5. Chamberlain, L.H., et al. 2000. Cysteine-string protein: the chaperone at the synapse. *J. Neurochem.* 74: 1781-1789.
6. Magga, J.M., et al. 2000. Cysteine string protein regulates G protein modulation of N-type calcium channels. *Neuron* 28: 195-204.
7. Gleave, T.L., et al. 2001. Cysteine string protein expression in mammary epithelial cells. *Pflugers Arch.* 441: 639-649.

CHROMOSOMAL LOCATION

Genetic locus: DNAJC5 (human) mapping to 20q13.33.

PRODUCT

CSP 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 CSP shRNA Plasmid (h): sc-43709-SH and CSP shRNA (h) Lentiviral Particles: sc-43709-V as alternate gene silencing products.

For independent verification of CSP (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-43709A, sc-43709B and sc-43709C.

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

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

CSP (H-3): sc-137128 is recommended as a control antibody for monitoring of CSP 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 CSP gene expression knockdown using RT-PCR Primer: CSP (h)-PR: sc-43709-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.