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# CSP shRNA (h) Lentiviral Particles: sc-43709-V

## 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  $\beta$ -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.

## CHROMOSOMAL LOCATION

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

## PRODUCT

CSP shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CSP siRNA (h): sc-43709 and CSP shRNA Plasmid (h): sc-43709-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ\text{C}$ . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ\text{C}$  for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

CSP shRNA (h) Lentiviral Particles is recommended for the inhibition of CSP expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## 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  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ\text{C}$  and the extension temperature should be  $68-72^\circ\text{C}$ .

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.