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# CAPON shRNA (h) Lentiviral Particles: sc-43660-V

## BACKGROUND

CAPON (carboxy-terminal PDZ ligand of nNOS) selectively binds within the 100 amino acid PDZ domain of the neuronal nitric oxide synthase (nNOS), but not to endothelial NOS or inducible NOS, and sequesters nNOS in the cytosol. Bio-synthesis of the neurotransmitter nitric oxide (NO) requires the association of nNOS with various synaptic proteins, including syntrophin, postsynaptic density (PSD)-95 and PSD-93 through a scaffolding PDZ domain. These proteins facilitate the transport of nNOS to the plasma membrane, where it is catalytically activated by NMDA-receptor mediated calcium channels. The association of nNOS with PSD-95 or PSD-93 is regulated by CAPON. The carboxy-terminus of CAPON binds to the PDZ domain, competes with PSD-95 and PSD-93 for binding to nNOS and in turn prevents the translocation and catalytic activation of nNOS.

## REFERENCES

1. Kornau, H.C., Schenker, L.T., Kennedy, M.B. and Seeburg P.H. 1995. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* 269: 1737-1740.
2. Stricker, N.L., Christopherson, K.S., Yi, B.A., Schatz, P.J., Raab, R.W., Dawes, G., Basset, D.E., Jr., Bredt, D.S. and Li, M. 1997. PDZ domain of neuronal nitric oxide synthase recognizes novel C-terminal peptide sequences. *Nat. Biotechnol.* 15: 336-342.
3. Jaffrey, S.R., Snowman, A.M., Eliasson, M.J., Cohen, N.A. and Snyder, S.H. 1998. CAPON: a protein associated with neuronal nitric oxide synthase that regulates its interactions with PSD-95. *Neuron* 20: 115-124.
4. Hashida-Okumura, A., Okumura, N., Iwamatsu, A., Buijs, R.M., Romijn, H.J. and Nagai, K. 1999. Interaction of neuronal nitric-oxide synthase with  $\alpha$ 1-syntrophin in rat brain. *J. Biol. Chem.* 274: 11736-11741.

## CHROMOSOMAL LOCATION

Genetic locus: NOS1AP (human) mapping to 1q23.3.

## PRODUCT

CAPON 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 CAPON siRNA (h): sc-43660 and CAPON shRNA Plasmid (h): sc-43660-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

CAPON shRNA (h) Lentiviral Particles is recommended for the inhibition of CAPON 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

CAPON (R-300): sc-9138 is recommended as a control antibody for monitoring of CAPON 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CAPON gene expression knockdown using RT-PCR Primer: CAPON (h)-PR: sc-43660-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.