SANTA CRUZ BIOTECHNOLOGY, INC.

CAPON siRNA (m): sc-142003

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. Biosynthesis 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 α1-syntrophin in rat brain. J. Biol. Chem. 274: 11736-11741.

CHROMOSOMAL LOCATION

Genetic locus: Nos1ap (mouse) mapping to 1 H3.

PRODUCT

CAPON 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CAPON shRNA Plasmid (m): sc-142003-SH and CAPON shRNA (m) Lentiviral Particles: sc-142003-V as alternate gene silencing products.

For independent verification of CAPON (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-142003A, sc-142003B and sc-142003C.

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 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

CAPON siRNA (m) is recommended for the inhibition of CAPON 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 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

CAPON (C-9): sc-374504 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 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 CAPON gene expression knockdown using RT-PCR Primer: CAPON (m)-PR: sc-142003-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.