

NMDA ϵ 4 siRNA (m): sc-36088

BACKGROUND

Glutamate receptors mediate most excitatory neurotransmission in the brain and play an important role in neural plasticity, neural development and neurodegeneration. Ionotropic glutamate receptors are categorized into NMDA receptors and kainate/AMPA receptors, both of which contain glutamate-gated, cation-specific ion channels. Kainate/AMPA receptors are co-localized with NMDA receptors in many synapses and consist of seven structurally related subunits designated GluR-1 to -7. The kainate/AMPA receptors are primarily responsible for fast excitatory neurotransmission by glutamate, whereas the NMDA receptors exhibit slow kinetics of Ca²⁺ ions and a high permeability for Ca²⁺ ions. The NMDA receptors consist of five subunits: ϵ 1, 2, 3, 4 and one ζ subunit. The ζ subunit is expressed throughout the brainstem whereas the four ϵ subunits display limited distribution.

REFERENCES

- Choi, D.W., et al. 1990. The role of glutamate neurotoxicity in hypoxic-ischemic neuronal death. *Annu. Rev. Neurosci.* 13: 171-182.
- Nakanishi, S. 1992. Molecular diversity of glutamate receptors and implications for brain function. *Science* 258: 597-603.
- Stern, P., et al. 1992. Fast and slow components of unitary EPSCs on stellate cells elicited by focal stimulation in slices of rat visual cortex. *J. Physiol.* 449: 247-278.
- Bliss, T.V., et al. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31-39.
- Watanabe, M., et al. 1994. Distinct distributions of five NMDA receptor channel subunit mRNAs in the brainstem. *J. Comp. Neurol.* 343: 520-531.
- Hollmann, M., et al. 1994. Cloned glutamate receptors. *Annu. Rev. Neurosci.* 17: 31-108.
- Schiffer, H.H., et al. 1997. Rat GluR7 and a carboxy-terminal splice variant, GluR7 β are functional kainate receptor subunits with a low sensitivity to glutamate. *Neuron* 19: 1141-1146.

CHROMOSOMAL LOCATION

Genetic locus: Grin2d (mouse) mapping to 7 B4.

PRODUCT

NMDA ϵ 4 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 NMDA ϵ 4 shRNA Plasmid (m): sc-36088-SH and NMDA ϵ 4 shRNA (m) Lentiviral Particles: sc-36088-V as alternate gene silencing products.

For independent verification of NMDA ϵ 4 (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-36088A, sc-36088B and sc-36088C.

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

NMDA ϵ 4 siRNA (m) is recommended for the inhibition of NMDA ϵ 4 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

NMDA ϵ 4 (G-10): sc-17822 is recommended as a control antibody for monitoring of NMDA ϵ 4 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 NMDA ϵ 4 gene expression knockdown using RT-PCR Primer: NMDA ϵ 4 (m)-PR: sc-36088-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.