

NMDAε2 siRNA (r): sc-270104

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 Ca^{2+} ions and a high permeability for Ca^{2+} 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

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- 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. *Ann. 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: Grin2b (rat) mapping to 4q43.

PRODUCT

NMDAε2 siRNA (r) 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 NMDAε2 shRNA Plasmid (r): sc-270104-SH and NMDAε2 shRNA (r) Lentiviral Particles: sc-270104-V as alternate gene silencing products.

For independent verification of NMDAε2 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270104A, sc-270104B and sc-270104C.

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

NMDAε2 siRNA (r) is recommended for the inhibition of NMDAε2 expression in rat 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

NMDAε2 (A-8): sc-365597 is recommended as a control antibody for monitoring of NMDAε2 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 NMDAε2 gene expression knockdown using RT-PCR Primer: NMDAε2 (r)-PR: sc-270104-PR (20 μl , 540 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

RESEARCH USE

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