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EAAT4 siRNA (h): sc-41942

BACKGROUND

Excitatory Amino Acid Transporters (EAATs) are membrane-bound proteins that are localized in glial cells and pre-synaptic glutamatergic nerve endings. EAATs transport the excitatory neurotransmitters L-glutamate and D-aspartate, a process that is essential for terminating the postsynaptic action of glutamate. The re-uptake of amino acid neurotransmitters by EAAT proteins has been shown to protect neurons from excitotoxicity, which is caused by the accumulation of amino acid neurotransmitters. EAAT4 is an aspartate/glutamate transporter that is expressed predominantly in the cerebellum. The transport activity encoded by EAAT4 has high apparent affinity for L-aspartate and L-glutamate, and has a pharmacologic profile consistent with previously described cerebellar transport activities. EAAT5 is a glutamate transporter coupled to a chloride conductance which is expressed primarily in retina. Although EAAT5 shares the structural homologies of the EAAT family, a novel feature of the EAAT5 sequence is a carboxy-terminal motif previously identified in N-ethyl-D-aspartate receptors and potassium channels and shown to confer interactions with a family of synaptic proteins that promote ion channel clustering.

REFERENCES

1. Arriza, J.L., et al. 1994. Functional comparisons of three glutamate transporter subtypes cloned from human motor cortex. *J. Neurosci.* 14: 5559-5569.
2. Fairman, W.A., et al. 1995. An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. *Nature* 375: 599-603.
3. Ikeda, J., et al. 1996. Nuclear disintegration as a leading step of glutamate excitotoxicity in brain neurons. *J. Neurosci. Res.* 43: 613-622.
4. Arriza, J.L., et al. 1997. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. *Proc. Natl. Acad. Sci. USA* 94: 4155-4160.
5. Rauen, T., et al. 1998. High-affinity glutamate transporters in the rat retina: a major role of the glial glutamate transporter GLAST-1 in transmitter clearance. *Cell Tissue Res.* 291: 19-31.
6. Jackson, M., et al. 2001. Modulation of the neuronal glutamate transporter EAAT4 by two interacting proteins. *Nature* 410: 89-93.

CHROMOSOMAL LOCATION

Genetic locus: SLC1A6 (human) mapping to 19p13.12.

PRODUCT

EAAT4 siRNA (h) 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 EAAT4 shRNA Plasmid (h): sc-41942-SH and EAAT4 shRNA (h) Lentiviral Particles: sc-41942-V as alternate gene silencing products.

For independent verification of EAAT4 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41942A, sc-41942B and sc-41942C.

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

EAAT4 siRNA (h) is recommended for the inhibition of EAAT4 expression in human 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

EAAT4 (6D9): sc-293344 is recommended as a control antibody for monitoring of EAAT4 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 EAAT4 gene expression knockdown using RT-PCR Primer: EAAT4 (h)-PR: sc-41942-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.