

EAAT2 siRNA (r): sc-270106

BACKGROUND

Excitatory amino acid transporter 1 (EAAT1) is one of the two glial glutamate transporters that clear the extracellular glutamate generated during neuronal signal transmission. 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 reuptake 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. Three glutamate transporters have been identified in human brain, designated EAAT1-3. EAAT1 and EAAT3 are also expressed in various non-nervous tissues, while EAAT2 expression appears to be restricted to the brain. Surface expression of the glial glutamate transporter EAAT1 is stimulated by Insulin-like growth factor 1 through activation of phosphatidylinositol-3-kinase.

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. Kirschner, M.A., et al. 1994. Mouse excitatory amino acid transporter EAAT2: isolation, characterization, and proximity to neuroexcitability loci on mouse chromosome 2. *Genomics* 24: 218-224.
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. Stoffel, W., et al. 1996. Human high affinity, Na⁺-dependent L-glutamate/L-aspartate transporter GLAST-1 (EAAT-1): gene structure and localization to chromosome 5p11-p12. *FEBS Lett.* 386: 189-193.
5. Sutherland, M.L., et al. 1996. Glutamate transporter mRNA expression in proliferative zones of the developing and adult murine CNS. *J. Neurosci.* 16: 2191-2207.
6. 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.

CHROMOSOMAL LOCATION

Genetic locus: Slc1a2 (rat) mapping to 3q31.

PRODUCT

EAAT2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EAAT2 shRNA Plasmid (r): sc-270106-SH and EAAT2 shRNA (r) Lentiviral Particles: sc-270106-V as alternate gene silencing products.

For independent verification of EAAT2 (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-270106A, sc-270106B and sc-270106C.

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

EAAT2 siRNA (r) is recommended for the inhibition of EAAT2 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 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

EAAT2 (E-1): sc-365634 is recommended as a control antibody for monitoring of EAAT2 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 EAAT2 gene expression knockdown using RT-PCR Primer: EAAT2 (r)-PR: sc-270106-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.