

EAAT1 siRNA (h): sc-35253

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. 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.

CHROMOSOMAL LOCATION

Genetic locus: SLC1A3 (human) mapping to 5p13.2.

PRODUCT

EAAT1 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 EAAT1 shRNA Plasmid (h): sc-35253-SH and EAAT1 shRNA (h) Lentiviral Particles: sc-35253-V as alternate gene silencing products.

For independent verification of EAAT1 (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-35253A, sc-35253B and sc-35253C.

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

EAAT1 siRNA (h) is recommended for the inhibition of EAAT1 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.

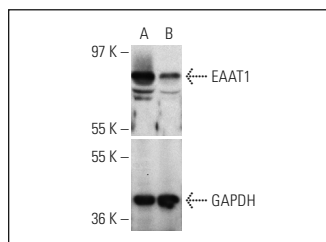
GENE EXPRESSION MONITORING

EAAT1 (A-3): sc-515839 is recommended as a control antibody for monitoring of EAAT1 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EAAT1 gene expression knockdown using RT-PCR Primer: EAAT1 (h)-PR: sc-35253-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

DATA



EAAT1 siRNA (h): sc-35253. Western blot analysis of EAAT1 expression in non-transfected control (A) and EAAT1 siRNA transfected (B) HeLa cells. Blot probed with EAAT1 (H-50): sc-15316. GAPDH (FL-335): sc-25778 used as specificity and loading control.

SELECT PRODUCT CITATIONS

1. Lee, S.G., et al. 2008. Mechanism of ceftriaxone induction of excitatory amino acid transporter-2 expression and glutamate uptake in primary human astrocytes. *J. Biol. Chem.* 283: 13116-13123.

RESEARCH USE

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