

GlyT2 siRNA (h): sc-41976

BACKGROUND

Na⁺/Cl⁻ dependent neurotransmitter transporters are a superfamily of transmembrane proteins that contain 12 membrane spanning regions. Specifically, the highly hydrophobic Na⁺/Cl⁻ dependent glycine transporters (GlyT) are crucial for the termination of neurotransmission at glycinergic synapses. Two different GlyT genes encode GlyT2 and GlyT1, which exists as two isoforms produced by alternative splicing of the same gene located on human chromosome 1p31.3. The GlyT1 gene may be an early marker of neural development and encodes glia-specific transporter proteins. Although GlyT1 and GlyT2 are both expressed in the brain and spinal cord, each shows a unique pattern of expression. GlyT1 is found only in the white matter of the CNS, whereas GlyT2 is found in the gray matter of the CNS as well as in macrophages and mast cells in peripheral tissues. The anatomic distribution of GlyT2 mRNA suggests that glycine may act as a supraspinal neurotransmitter and may function as a chemical messenger outside the CNS.

REFERENCES

1. Liu, Q.R., et al. 1992. Cloning and expression of a glycine transporter from mouse brain. *FEBS Lett.* 305: 110-114.
2. Borowsky, B., et al. 1993. Two glycine transporter variants with distinct localization in the CNS and peripheral tissues are encoded by a common gene. *Neuron* 10: 851-863.
3. Kim, K.M., et al. 1994. Cloning of the human glycine transporter type 1: molecular and pharmacological characterization of novel isoform variants and chromosomal localization of the gene in the human and mouse genomes. *Mol. Pharmacol.* 45: 608-617.
4. Adams, R.H., et al. 1995. Gene structure and glial expression of the glycine transporter GlyT1 in embryonic and adult rodents. *J. Neurosci.* 15: 2524-2532.
5. Evans, J., et al. 1999. Cloning, functional characterisation and population analysis of a variant form of the human glycine type 2 transporter. *FEBS Lett.* 463: 301-306.
6. Horiuchi, M., et al. 2001. Surface-localized glycine transporters 1 and 2 function as monomeric proteins in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* 98: 1448-1453.

CHROMOSOMAL LOCATION

Genetic locus: SLC6A5 (human) mapping to 11p15.1.

PRODUCT

GlyT2 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 GlyT2 shRNA Plasmid (h): sc-41976-SH and GlyT2 shRNA (h) Lentiviral Particles: sc-41976-V as alternate gene silencing products.

For independent verification of GlyT2 (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-41976A, sc-41976B and sc-41976C.

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

GlyT2 siRNA (h) is recommended for the inhibition of GlyT2 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

GlyT2 (B-4): sc-390090 is recommended as a control antibody for monitoring of GlyT2 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 GlyT2 gene expression knockdown using RT-PCR Primer: GlyT2 (h)-PR: sc-41976-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.