

GlyR α 1 siRNA (h): sc-42467

BACKGROUND

In the central nervous system (CNS), glycine-mediated inhibitory neurotransmission is essential to voluntary motor control and reflex responses. Glycine binds to glycine receptors (GlyR) in the post synaptic neuronal membranes. GlyR, γ -aminobutyric acid, serotonin and acetylcholine comprise an evolutionally conserved superfamily of ligand-gated ion channels. The pentameric subunit structure of GlyR consists of two types of glycosylated membrane proteins, α 1 through α 4 and β , and an associated peripheral membrane protein, which combine to form a chloride-selective ion channel. In humans, the composition of the pentamer changes from α 2 subunits in the fetal CNS to α 1 and β subunits in the adult CNS. Fast potentiation of GlyR by intracellular Ca^{2+} in the brainstem and midbrain indicate an important role for Ca^{2+} in modulation glycinergic synapses. The genes encoding human GlyR α 1, α 2, α 3 and β subunits map to chromosomes 5q33.1, Xp22.2, 4q34.1 and 4q32.1, respectively.

REFERENCES

1. Pfeiffer, F., et al. 1981. Solubilisation of the glycine receptor from rat spinal cord. *Brain Res.* 226: 273-279.
2. Pfeiffer, F., et al. 1982. Purification by affinity chromatography of the glycine receptor of rat spinal cord. *J. Biol. Chem.* 257: 9389-9393.
3. Genningsloh, G., et al. 1987. The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. *Nature* 328: 215-220.
4. Schofield, P.R., et al. 1987. Sequence and functional expression of the GABA_A receptor shows a ligand-gated receptor super-family. *Nature* 328: 221-227.
5. Langosch, D., et al. 1988. Conserved quaternary structure of ligand-gated ion channels: the postsynaptic glycine receptor is a pentamer. *Proc. Natl. Acad. Sci. USA* 85: 7394-7398.
6. Hoch, W., et al. 1989. Primary cultures of mouse spinal cord express the neonatal isoform of the inhibitory glycine receptor. *Neuron* 3: 339-348.
7. Genningsloh, G., et al. 1990. α subunit variants of the human glycine receptor: primary structures, functional expression and chromosomal location of corresponding genes. *EMBO J.* 9: 771-776.

CHROMOSOMAL LOCATION

Genetic locus: GLRA1 (human) mapping to 5q33.1.

PRODUCT

GlyR α 1 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 GlyR α 1 shRNA Plasmid (h): sc-42467-SH and GlyR α 1 shRNA (h) Lentiviral Particles: sc-42467-V as alternate gene silencing products.

For independent verification of GlyR α 1 (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-42467A, sc-42467B and sc-42467C.

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

GlyR α 1 siRNA (h) is recommended for the inhibition of GlyR α 1 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

GlyR α 1 (2E7): sc-293498 is recommended as a control antibody for monitoring of GlyR α 1 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 GlyR α 1 gene expression knockdown using RT-PCR Primer: GlyR α 1 (h)-PR: sc-42467-PR (20 μ l, 542 bp). 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.