SANTA CRUZ BIOTECHNOLOGY, INC.

GlyR β siRNA (m): sc-42472

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

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 postsynaptic neuronal membranes. GlyR, γ-aminobutryic 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, α1 through α4 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 β2 subunits in the fetal CNS to α1 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 of glycinergic synapses.

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. Genningloh, 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 quarternary structure of ligand-gated ion channels: the postsynaptic glycine receptor is a pentameter. 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 recpetor. Neuron 3: 339-348.
7. Grenningloh, 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: Glrb (mouse) mapping to 3 E3.

PRODUCT

GlyR β siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GlyR β shRNA Plasmid (m): sc-42472-SH and GlyR β shRNA (m) Lentiviral Particles: sc-42472-V as alternate gene silencing products.

For independent verification of GlyR β (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-42472A, sc-42472B and sc-42472C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GlyR β siRNA (m) is recommended for the inhibition of GlyR β expression in mouse 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 µM in 66 µl. 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 β (D-8): sc-365819 is recommended as a control antibody for monitoring of GlyR β 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 β gene expression knockdown using RT-PCR Primer: GlyR β (m)-PR: sc-42472-PR (20 µl). 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.