SANTA CRUZ BIOTECHNOLOGY, INC.

GlyRS siRNA (m): sc-75154

BACKGROUND

The fidelity of protein synthesis requires efficient discrimination of amino acid substrates by aminoacyl-tRNA synthetases. Proteins belonging to this family function to catalyze the aminoacylation of tRNAs by their corresponding amino acids, thus linking amino acids with tRNA-contained nucleotide triplets. GlyRS (Glycyl-tRNA synthetase), also known as Glycine-tRNA ligase, is a 739 amino acid class II synthetase that is widely expressed, including in the brain and spinal cord. Defects in the gene encoding GlyRS is the cause of Charcot-Marie-Tooth disease type 2D (CMT2D), which is an autosomal dominant inherited disease characterized by severe weakness, atrophy and absence of deep tendon reflexes in the upper extremities. Defects in the GlyRS gene is also the cause of distal hereditary muscular neuropathy type V (HMN5), a disease similar to CMT2D, though the distal sensory involvement is less severe in HMN5 patients.

REFERENCES

1. Shiba, K., et al. 1994. Human glycyl-tRNA synthetase. Wide divergence of primary structure from bacterial counterpart and species-specific aminoacylation. J. Biol. Chem. 269: 30049-30055.
2. Williams, J., et al. 1995. Cloning, sequencing and bacterial expression of human glycine tRNA synthetase. Nucleic Acids Res. 23: 1307-1310.
3. Antonellis, A., et al. 2003. Glycyl tRNA synthetase mutations in Charcot-Marie-Tooth disease type 2D and distal spinal muscular atrophy type V. Am. J. Hum. Genet. 72: 1293-1299.
4. Antonellis, A., et al. 2006. Functional analyses of glycyl-tRNA synthetase mutations suggest a key role for tRNA-charging enzymes in peripheral axons. J. Neurosci. 26: 10397-10406.
5. James, P.A., et al. 2006. Severe childhood SMA and axonal CMT due to anticodon binding domain mutations in the GARS gene. Neurology 67: 1710-1712.
6. Scherer, S.S. 2006. Inherited neuropathies: new genes don't fit old models. Neuron 51: 672-674.
7. Seburn, K.L., et al. 2006. An active dominant mutation of glycyl-tRNA synthetase causes neuropathy in a Charcot-Marie-Tooth 2D mouse model. Neuron 51: 715-726.

CHROMOSOMAL LOCATION

Genetic locus: Gars (mouse) mapping to 6 B3.

PRODUCT

GlyRS 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 GlyRS shRNA Plasmid (m): sc-75154-SH and GlyRS shRNA (m) Lentiviral Particles: sc-75154-V as alternate gene silencing products.

For independent verification of GlyRS (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-75154A, sc-75154B and sc-75154C.

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

GlyRS siRNA (m) is recommended for the inhibition of GlyRS 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

GlyRS (D-10): sc-365311 is recommended as a control antibody for monitoring of GlyRS 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 GlyRS gene expression knockdown using RT-PCR Primer: GlyRS (m)-PR: sc-75154-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.