SANTA CRUZ BIOTECHNOLOGY, INC.

3PGDH siRNA (h): sc-105011

BACKGROUND

The survival and development of central neurons require the supply of trophic factors by glial cells. The trophic actions of glial cells on Purkinje neurons are mediated by L-serine and glycine, which are glia-derived trophic factors synthesized by 3PGDH. 3PGDH protein is 544 amino acids in length. Two distinct mRNA transcripts that encode for 3PGDH protein in normal human tissues are dominant 2.1 kb mRNA, which is highly expressed in prostate, testis, ovary, brain, liver, kidney and pancreas, and weakly expressed in thymus, colon and heart, and 710 bp mRNA, which is highly expressed in heart and skeletal muscle. 3PGDH is regulated at the transcriptional level depending on tissue specificity and cellular proliferative status. 3PGDH protein is also highly expressed in adult and fetal brain tissues. 3PGDH protein plays an important role in the metabolism, development and function of the central nervous system and its deficiency is a treatable congential error that impairs L-serine biosynthesis which is characterized by congenital microcephaly, psychomotor retardation and seizures.

REFERENCES

1. de Koning, T.J., et al. 1998. Beneficial effects of L-serine and glycine in the management of seizures in 3-phosphoglycerate dehydrogenase deficiency. Ann. Neurol. 44: 261-265.
2. Shigeki, F., et al. 2000. L-serine and glycine serve as major astroglia-derived trophic factors for cerebellar Purkinje neurons. Proc. Natl. Acad. Sci. USA 97: 11528-11533.
3. Cho, H.M., et al. 2000. Nucleotide sequence and differential expression of the human 3-phosphoglycerate dehydrogenase gene. Gene 245: 193-201.
4. Klomp, L.W., et al. 2000. Molecular characterization of 3-phosphoglycerate dehydrogenase deficiency—a neurometabolic disorder associated with reduced L-serine biosynthesis. Am. J. Hum. Genet. 67: 1389-1399.
5. Pineda, M., et al. 2000. 3-phosphoglycerate dehydrogenase deficiency in a patient with West syndrome. Dev. Med. Child. Neurol. 42: 629-633.

CHROMOSOMAL LOCATION

Genetic locus: PHGDH (human) mapping to 1p12.

PRODUCT

3PGDH 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see 3PGDH shRNA Plasmid (h): sc-105011-SH and 3PGDH shRNA (h) Lentiviral Particles: sc-105011-V as alternate gene silencing products.

For independent verification of 3PGDH (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-105011A, sc-105011B and sc-105011C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

3PGDH siRNA (h) is recommended for the inhibition of 3PGDH 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 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

3PGDH (B-1): sc-390610 is recommended as a control antibody for monitoring of 3PGDH 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 3PGDH gene expression knockdown using RT-PCR Primer: 3PGDH (h)-PR: sc-105011-PR (20 µl, 429 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.