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GSS siRNA (m): sc-41981

BACKGROUND

GSS (glutathione synthetase) is a 474 amino acid protein encoded by the gene located at human chromosome 20q11.2. GSS consists of three loops projecting from an antiparallel β -sheet, a parallel β -sheet and a lid of anti-parallel sheets, which provide access to the ATP-binding site. Although Southern blot and gene analysis suggest that GSS may be the only member of a unique family, the crystal structure indicates that GSS belongs to the ATP-GRASP superfamily. GSS is expressed in hemocytes and nucleated cells, including the brain. GSS occurs as a homodimer. There are two steps in the production of Glutathione, beginning with γ -GCS and ending with GSS. In an ATP-dependent reaction, GSS produces Glutathione from γ -glutamylcysteine and glycine precursors. Partial hepatectomy, diethyl maleate, buthionine sulfoximine, tert-butylhydroquinone and thioacetamide increase the expression of GSS, which causes an increase in Glutathione levels. An inherited autosomal recessive disorder, 5-oxoprolinuria (pyroglutamic aciduria), is caused by GSS deficiencies, which leads to central nervous system damage, hemolytic anemia, metabolic acidosis and urinary excretion of 5-oxoproline. A missense mutation in the gene encoding GSS leads to a GSS deficiency restricted to erythrocytes, which causes only hemolytic anemia.

REFERENCES

1. Webb, G.C., Vaska, V.L., Gali, R.R., Ford, J.H. and Board, P.G. 1995. The gene encoding human Glutathione synthetase (GSS) maps to the long arm of chromosome 20 at band 11.2. *Genomics* 30: 617-619.
2. Gali, R.R. and Board, P.G. 1995. Sequencing and expression of a cDNA for human Glutathione synthetase. *Biochem. J.* 310: 353-358
3. Shi, Z.Z., Habib, G.M., Rhead, W.J., Gahl, W.A., He, X., Sazer, S. and Lieberman, M.W. 1996. Mutations in the Glutathione synthetase gene cause 5-oxoprolinuria. *Nat. Genet.* 14: 361-365.
4. Polekhina, G., Board, P.G., Gali, R.R., Rossjohn, J. and Parker, M.W. 1999. Molecular basis of Glutathione synthetase deficiency and a rare gene permutation event. *EMBO J.* 18: 3204-3213.
5. Huang, Z.A., Yang, H., Chen, C., Zeng, Z. and Lu, S.C. 2000. Inducers of γ -glutamylcysteine synthetase and their effects on Glutathione synthetase expression. *Biochim. Biophys. Acta* 1493: 48-55.

CHROMOSOMAL LOCATION

Genetic locus: Gss (mouse) mapping to 2 H1.

PRODUCT

GSS 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GSS shRNA Plasmid (m): sc-41981-SH and GSS shRNA (m) Lentiviral Particles: sc-41981-V as alternate gene silencing products.

For independent verification of GSS (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-41981A, sc-41981B and sc-41981C.

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

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

GSS (H-7): sc-166882 is recommended as a control antibody for monitoring of GSS 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 GSS gene expression knockdown using RT-PCR Primer: GSS (m)-PR: sc-41981-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.