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γ-GCSc siRNA (m): sc-41979

BACKGROUND

The GCLC gene consists of 16 exons and encodes the 636 amino acid protein γ-GCSc (γ-glutamylcysteine synthetase heavy subunit), also designated γ-L-glutamate-L-cysteine ligase catalytic subunit (GLCLC). γ-GCSc is expressed in hemocytes, brain, liver and kidney. γ-GCSc associates with a regulatory or modifier subunit, γ-GCScm (γ-glutamylcysteine synthetase light subunit), to form a heterodimer, γ-GCS. γ-GCS is the first enzyme involved and the rate determining step in glutathione biosynthesis. Oxidants, cadmium and methylmercury upregulate the transcription of γ-GCS. H₂O₂ regulation depends on the Yap1 protein and the presence of glutamate, glutamine and lysine. Cadmium regulates transcription through proteins Met-4, Met-31 and Met-32. Cbf1, a DNA binding protein, inhibits transcription of γ-GCS. Chemopreventive compounds cause increased levels of γ-GCSc in kidney tissues, which may protect against chemically induced carcinogenesis. A His370Leu amino acid change in γ-GCSc causes deficiencies in activity which are responsible for hemolytic anemia and low red blood cell glutathione levels.

REFERENCES

- Lunn, G., et al. 1979. Transport accounts for glutathione turnover in human erythrocytes. *Blood* 54: 238.
- Sierra-Rivera, E., et al. 1995. Assignment of the gene (GLCLC) that encodes the heavy subunit of γ-glutamylcysteine synthetase to human chromosome 6. *Cytogenet. Cell Genet.* 70: 278-279.
- Walsh, A.C., et al. 1996. Genetic mapping of GLCLC, the human gene encoding the catalytic subunit of γ-glutamyl-cysteine synthetase, to chromosome band 6p12 and characterization of a polymorphic trinucleotide repeat within its 5-prime untranslated region. *Cytogenet. Cell Genet.* 75: 14-16.

CHROMOSOMAL LOCATION

Genetic locus: *Gclc* (mouse) mapping to 9 E1.

PRODUCT

γ-GCSc 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 γ-GCSc shRNA Plasmid (m): sc-41979-SH and γ-GCSc shRNA (m) Lentiviral Particles: sc-41979-V as alternate gene silencing products.

For independent verification of γ-GCSc (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-41979A, sc-41979B and sc-41979C.

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

γ-GCSc siRNA (m) is recommended for the inhibition of γ-GCSc 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

γ-GCSc (H-5): sc-390811 is recommended as a control antibody for monitoring of γ-GCSc 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 γ-GCSc gene expression knockdown using RT-PCR Primer: γ-GCSc (m)-PR: sc-41979-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Jain, S.K., et al. 2016. L-cysteine supplementation upregulates glutathione (GSH) and vitamin D binding protein (VDBP) in hepatocytes cultured in high glucose and *in vivo* in liver, and increases blood levels of GSH, VDBP, and 25-hydroxy-vitamin D in Zucker diabetic fatty rats. *Mol. Nutr. Food Res.* 60: 1090-1098.

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.