SANTA CRUZ BIOTECHNOLOGY, INC.

γ-GCSc siRNA (h): sc-41978

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-GCSm (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, cadium and methyl mercury upregulate the transcription of γ-GCS. H_2O_2 regulation depends on the Yap1 protein and the presence of glutamate, glutamine and lysine. Cadium 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

1. Lunn, G., et al. 1979. Transport accounts for glutathione turnover in human erythrocytes. Blood 54: 238-244.
2. 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.

CHROMOSOMAL LOCATION

Genetic locus: GCLC (human) mapping to 6p12.1.

PRODUCT

γ-GCSc 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 γ-GCSc shRNA Plasmid (h): sc-41978-SH and γ-GCSc shRNA (h) Lentiviral Particles: sc-41978-V as alternate gene silencing products.

For independent verification of γ-GCSc (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-41978A, sc-41978B and sc-41978C.

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 (h) is recommended for the inhibition of γ-GCSc 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

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 (h)-PR: sc-41978-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

1. Jain, S.K., et al. 2015. Can L-cysteine and vitamin D rescue vitamin D and vitamin D binding protein levels in blood plasma of African American type 2 diabetic patients? Antioxid. Redox Signal. 23: 688-693.
2. Kanikarla-Marie, P. and Jain, S.K. 2016. 1,25$(OH)_2D_3$ inhibits oxidative stress and monocyte adhesion by mediating the upregulation of GCLC and GSH in endothelial cells treated with acetoacetate (ketosis). J. Steroid Biochem. Mol. Biol. 159: 94-101.
3. Parsanathan, R. and Jain, S.K. 2018. L-cysteine *in vitro* can restore cellular glutathione and inhibits the expression of cell adhesion molecules in G6PD-deficient monocytes. Amino Acids 50: 909-921.
4. Parsanathan, R. and Jain, S.K. 2019. Glutathione deficiency alters the vitamin D-metabolizing enzymes CYP27B1 and CYP24A1 in human renal proximal tubule epithelial cells and kidney of HFD-fed mice. Free Radic. Biol. Med. 131: 376-381.

RESEARCH USE

For research use only, not for use in diagnostic procedures.