

Produktinformation



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Diagnostik & molekulare Diagnostik



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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic in



γ-GCSc siRNA (h): sc-41978



The Power to Ouestion

BACKGROUND

The GCLC gene consists of 16 exons and encodes the 636 amino acid protein $\gamma\text{-GCSc}$ ($\gamma\text{-glutamylcysteine}$ synthetase heavy subunit), also designated $\gamma\text{-L-glutamate-L-cysteine}$ ligase catalytic subunit (GLCLC). $\gamma\text{-GCSc}$ is expressed in hemocytes, brain, liver and kidney. $\gamma\text{-GCSc}$ associates with a regulatory or modifier subunit, $\gamma\text{-GCSm}$ ($\gamma\text{-glutamylcysteine}$ synthetase light subunit), to form a heterodimer, $\gamma\text{-GCS}$. $\gamma\text{-GCS}$ is the first enzyme involved and the rate determining step in glutathione biosynthesis. Oxidants, cadium and methyl mercury upregulate the transcription of $\gamma\text{-GCS}$. 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 $\gamma\text{-GCS}$. Chemopreventive compounds cause increased levels of $\gamma\text{-GCSc}$ in kidney tissues, which may protect against chemically-induced carcinogenesis. A His370Leu amino acid change in $\gamma\text{-GCSc}$ 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.
- 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.
- 3. Walsh, A.C., et al. 1996. Genetic mapping of GLCLC, the human gene encoding the catalytic subunit of γ-glutamylcysteine synthetase, to chromosome band 6p12 and characterization of a polymorphic trinucleotide repeat within its 5' untranslated region. Cytogenet. Cell Genet. 75: 14-16.

CHROMOSOMAL LOCATION

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

PRODUCT

 $\gamma\text{-GCSc}$ 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 $\gamma\text{-GCSc}$ shRNA Plasmid (h): sc-41978-SH and $\gamma\text{-GCSc}$ 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 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

 $\gamma\text{-GCSc}$ siRNA (h) is recommended for the inhibition of $\gamma\text{-GCSc}$ 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-lgG κ BP-HRP: sc-516102 or m-lgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG κ BP-FITC: sc-516140 or m-lgG 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 γ -GCSc gene expression knockdown using RT-PCR Primer: γ -GCSc (h)-PR: sc-41978-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- 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.
- 2. 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.

PROTOCOLS

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

Santa Cruz Biotechnology, Inc. 1.800.457.3801 831.457.3801 Fax 831.457.3801 Europe +00800 4573 8000 49 6221 4503 0 www.scbt.com