

γ -GCSm siRNA (h): sc-40602

BACKGROUND

γ -glutamylcysteine synthetase (γ -GCS) is the rate limiting enzyme for glutathione (L- γ -glutamyl-L-cysteinylglycine, GSH) synthesis. GSH is ubiquitous in mammalian cells as a vital intra- and extracellular protective antioxidant. γ -GCS is a heterodimer of a heavy catalytic subunit and a light regulatory subunit that is responsive to inflammation, phenolic antioxidants, heat shock, oxidants and cytokines. The human γ -GCS gene encoding the 367 amino acid catalytic subunit maps to chromosome 6p12. The human γ -GCS gene encoding the regulatory subunit maps to chromosome 1p22-p21. The two subunits of γ -GCS form a heterodimeric zinc metalloprotein that gains activity through formation of a reversible disulfide bond.

REFERENCES

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CHROMOSOMAL LOCATION

Genetic locus: GCLM (human) mapping to 1p22.1.

PRODUCT

γ -GCSm 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see γ -GCSm shRNA Plasmid (h): sc-40602-SH and γ -GCSm shRNA (h) Lentiviral Particles: sc-40602-V as alternate gene silencing products.

For independent verification of γ -GCSm (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-40602A, sc-40602B and sc-40602C.

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

γ -GCSm siRNA (h) is recommended for the inhibition of γ -GCSm 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 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

γ -GCSm (E-4): sc-55586 is recommended as a control antibody for monitoring of γ -GCSm 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor γ -GCSm gene expression knockdown using RT-PCR Primer: γ -GCSm (h)-PR: sc-40602-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.