

# $\gamma$ -GCSm siRNA (m): sc-40603

## BACKGROUND

$\gamma$ -glutamylcysteine synthetase ( $\gamma$ -GCS) is the rate limiting enzyme for glutathione (L- $\gamma$ -glutamyl-L-cysteinylglycine, GSH) synthesis. GSH is ubiquitous in mammalian cells as a vital intra- and extracellular protective antioxidant.  $\gamma$ -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  $\gamma$ -GCS gene encoding the 367 amino acid catalytic subunit maps to chromosome 1p22.1. The human  $\gamma$ -GCS gene encoding the regulatory subunit maps to chromosome 1p22.1. The two subunits of  $\gamma$ -GCS form a heterodimeric zinc metalloprotein that gains activity through formation of a reversible disulfide bond.

## REFERENCES

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2. Anderson, M.E. 1998. Glutathione: an overview of biosynthesis and modulation. *Chem. Biol. Interact.* 111-112: 1-14.
3. Kondo, T., et al. 1999. Regulation of  $\gamma$ -glutamylcysteine synthetase expression in response to oxidative stress. *Free Radic. Res.* 31: 325-334.
4. Rahman, I. 1999. Inflammation and the regulation of glutathione level in lung epithelial cells. *Antioxid. Redox Signal.* 1: 425-447.
5. Rahman, I. and MacNee, W. 2000. Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. *Free Radic. Biol. Med.* 28: 1405-1420.
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7. Online Mendelian Inheritance in Man, OMIM<sup>™</sup>. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606857. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
8. LocusLink Report (LocusID: 2729). <http://www.ncbi.nlm.nih.gov/LocusLink/>

## CHROMOSOMAL LOCATION

Genetic locus: Gclm (mouse) mapping to 3 G1.

## PRODUCT

$\gamma$ -GCSm 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see  $\gamma$ -GCSm shRNA Plasmid (m): sc-40603-SH and  $\gamma$ -GCSm shRNA (m) Lentiviral Particles: sc-40603-V as alternate gene silencing products.

For independent verification of  $\gamma$ -GCSm (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-40603A, sc-40603B and sc-40603C.

## RESEARCH USE

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

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

$\gamma$ -GCSm siRNA (m) is recommended for the inhibition of  $\gamma$ -GCSm 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  $\mu$ M in 66  $\mu$ 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

$\gamma$ -GCSm (E-4): sc-55586 is recommended as a control antibody for monitoring of  $\gamma$ -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  $\gamma$ -GCSm gene expression knockdown using RT-PCR Primer:  $\gamma$ -GCSm (m)-PR: sc-40603-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.