

Grx1 siRNA (h): sc-72089

BACKGROUND

Glutaredoxin (Grx) and a relative, thioredoxin, catalyze general thiol-disulfide oxidoreductions and act as hydrogen donors for ribonucleotide reductase, an enzyme essential for DNA synthesis. Proteins which catalyze thiol-disulfide exchange reactions are required for electron and proton transport to essential enzymes like ribonucleotide reductase, for the formation of disulfide bonds during protein folding, and for general regulation of protein function by thiol redox control. These proteins also play a role in cellular defense against oxidative stress. The thioredoxin superfamily includes a number of proteins with the same basic folding and structure as thioredoxin and glutaredoxin, with the active site at the C-terminal end of a β -strand followed by an α -helix. Glutaredoxin (Grx) operates in thiol-disulfide reactions via two vicinal (CXYC) active site cysteine residues, which either form a disulfide (oxidized form) or a dithiol (reduced form). Mammalian cells contain at least two dithiol glutaredoxins: Grx1, the cytoplasmic form; and Grx2, which has mitochondrial and nuclear isoforms. Nuclear Grx2, unlike Grx1, is a substrate for thioredoxin reductase and has a higher affinity for S-glutathionylated proteins.

CHROMOSOMAL LOCATION

Genetic locus: GLRX (human) mapping to 5q15.

PRODUCT

Grx1 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 Grx1 shRNA Plasmid (h): sc-72089-SH and Grx1 shRNA (h) Lentiviral Particles: sc-72089-V as alternate gene silencing products.

For independent verification of Grx1 (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-72089A, sc-72089B and sc-72089C.

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

Grx1 siRNA (h) is recommended for the inhibition of Grx1 expression in human cells.

PROTOCOLS

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

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

Grx1 (3C11): sc-293250 is recommended as a control antibody for monitoring of Grx1 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG 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 Grx1 gene expression knockdown using RT-PCR Primer: Grx1 (h)-PR: sc-72089-PR (20 μ l, 586 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lee, S.O., et al. 2014. The orphan nuclear receptor NR4A1 (Nur77) regulates oxidative and endoplasmic reticulum stress in pancreatic cancer cells. *Mol. Cancer Res.* 12: 527-538.
2. Liu, X., et al. 2015. Glutaredoxin 1 (Grx1) protects human retinal pigment epithelial cells from oxidative damage by preventing Akt glutathionylation. *Invest. Ophthalmol. Vis. Sci.* 56: 2821-2832.
3. Liu, X., et al. 2016. The novel triterpenoid RTA 408 protects human retinal pigment epithelial cells against H₂O₂-induced cell injury via NF-E2-related factor 2 (Nrf2) activation. *Redox Biol.* 8: 98-109.
4. Liu, X., et al. 2016. Salvianolic acid B (Sal B) protects retinal pigment epithelial cells from oxidative stress-induced cell death by activating glutaredoxin 1 (Grx1). *Int. J. Mol. Sci.* 17: 1835.

RESEARCH USE

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