

GPx-4 siRNA (m): sc-63302

BACKGROUND

Glutathione peroxidase (GPx) enzymes are generally selenium-containing tetrameric glycoproteins that help prevent lipid peroxidation of cell membranes. GPx enzymes reduce lipid hydroperoxides to alcohols, and reduce free hydrogen peroxide to water. GPx members are among the few proteins known in higher vertebrates to contain selenocysteine, which occurs at the active site of glutathione peroxidase and is coded by the nonsense (stop) codon TGA. There are eight GPx homologs (GPx-1–8). GPx-1, Gpx-2 and Gpx-3 exist as homotetramers. Gpx-4 has a high tendency to form high molecular weight oligomers. GPx-1 plays an important role in the antioxidant defense of the vascular wall and neural cells in response to oxidative stress. GPx-2 is the major isoform in the lungs and its basal or inducible expression is dependent on Nrf2. GPx-3 is under regulation by hypoxic stress and the expression and deficiency of GPx-3 is associated with cardiovascular disease and stroke. GPx-5 is selenium-independent; it is bound to the acrosome of sperm, where it may protect sperm from premature acrosome reaction in the epididymis.

REFERENCES

1. Arai, M., et al. 1996. Import into mitochondria of phospholipid hydroperoxide glutathione peroxidase requires a leader sequence. *Biochem. Biophys. Res. Commun.* 227: 433-439.
2. Chu, F.F., et al. 1997. Expression and chromosomal mapping of mouse Gpx2 gene encoding the gastrointestinal form of glutathione peroxidase, GPX-GI. *Biomed. Environ. Sci.* 10: 156-162.

CHROMOSOMAL LOCATION

Genetic locus: Gpx4 (mouse) mapping to 10 C1.

PRODUCT

GPx-4 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GPx-4 shRNA Plasmid (m): sc-63302-SH and GPx-4 shRNA (m) Lentiviral Particles: sc-63302-V as alternate gene silencing products.

For independent verification of GPx-4 (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-63302A, sc-63302B and sc-63302C.

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

GPx-4 siRNA (m) is recommended for the inhibition of GPx-4 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 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

GPx-4 (E-12): sc-166570 is recommended as a control antibody for monitoring of GPx-4 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 GPx-4 gene expression knockdown using RT-PCR Primer: GPx-4 (m)-PR: sc-63302-PR (20 μ l, 571 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Li, L., et al. 2018. Ferroptosis is associated with oxygen-glucose deprivation/reoxygenation-induced Sertoli cell death. *Int. J. Mol. Med.* 41: 3051-3062.

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.