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GPx-4 siRNA (h): sc-44465

BACKGROUND

GPx-4, also known as phospholipid hydroperoxide glutathione peroxidase (PHGPx), is the only known antioxidant enzyme that reduces phospholipid hydroperoxides within membranes and lipoproteins, thus inhibiting lipid peroxidation. A number of pathophysiological states rely on peroxidation of lipids, suggesting that GPx-4 plays a crucial role in antioxidative defense. GPx-4 is expressed at low levels in a wide variety of organs with two distinct forms: L-GPx-4, which localizes in the mitochondria, and S-GPx-4, the cytosolic form. In some tissues, GPx-4 is more highly expressed, suggesting that GPx-4 is involved in more specific functions. For example, regulation of the enzyme in testicular tissue implies a necessary role for GPx-4 in sperm maturation. The gene encoding GPx-4 presents a number of different protein-binding domains, allowing regulation of expression to be influenced by Sp1, NF-Y and ApoER2, as well as other proteins. Therefore, complex interactions between a variety of proteins and the GPx-4 gene, in addition to interplay with fatty acids, cytokines and antioxidants, ultimately dictate the functional significance of GPx-4.

REFERENCES

1. Sneddon, A.A., et al. 2003. Regulation of selenoprotein GPx4 expression and activity in human endothelial cells by fatty acids, cytokine and antioxidants. *Atherosclerosis* 171: 57-65.
2. Ufer, C., et al. 2003. Functional characterization of *cis*- and *trans*-regulatory elements involved in expression of phospholipid hydroperoxide glutathione peroxidase. *Nucleic Acids Res.* 31: 4293-4303.
3. Zhao, L., et al. 2003. L-PHGPx expression can be suppressed by antisense oligonucleotides. *Arch. Biochem. Biophys.* 417: 212-218.
4. Andersen, O.M., et al. 2003. Essential role of apolipoprotein E receptor-2 in sperm development. *J. Biol. Chem.* 278: 23989-23995.

CHROMOSOMAL LOCATION

Genetic locus: GPX4 (human) mapping to 19p13.3.

PRODUCT

GPx-4 siRNA (h) is a pool of 2 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 (h): sc-44465-SH and GPx-4 shRNA (h) Lentiviral Particles: sc-44465-V as alternate gene silencing products.

For independent verification of GPx-4 (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-44465A and sc-44465B.

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.

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 (h) is recommended for the inhibition of GPx-4 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

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 (h)-PR: sc-44465-PR (20 μ l, 506 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.