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GPx-4 shRNA (h) Lentiviral Particles: sc-44465-V

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. 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.
3. Gladyshev, V.N., et al. 1999. Levels of major selenoproteins in T cells decrease during HIV infection and low molecular mass selenium compounds increase. *Proc. Natl. Acad. Sci. USA* 96: 835-839.

CHROMOSOMAL LOCATION

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

PRODUCT

GPx-4 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see GPx-4 siRNA (h): sc-44465 and GPx-4 shRNA Plasmid (h): sc-44465-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

GPx-4 shRNA (h) Lentiviral Particles is recommended for the inhibition of GPx-4 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

GPx-4 (B-12): sc-166120 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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