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Glutathione reductase shRNA (h) Lentiviral Particles: sc-44843-V

BACKGROUND

Glutathione reductase, also designated Glutathione reductase mitochondrial precursor, GRase, GSR or GR, belongs to the class-I pyridine nucleotide-disulfide oxidoreductase family. The main function of the protein is to maintain high levels of reduced glutathione in the cytosol. With the concomitant oxidation of NADPH, Glutathione reductase transforms oxidized glutathione to the reduced form. Glutathione reductase, which can localize to mitochondria or to the cytoplasm, can form a disulfide-linked homodimer. The active site of the protein is a redox-active disulfide bond.

REFERENCES

1. Staal, G.E., et al. 1969. Purification and properties of an abnormal Glutathione reductase from human erythrocytes. *Biochim. Biophys. Acta* 185: 63-69.
2. Karplus, P.A., et al. 1987. Refined structure of Glutathione reductase at 1.54 Å resolution. *J. Mol. Biol.* 195: 701-729.
3. Stoll, V.S., et al. 1997. Glutathione reductase turned into trypanothione reductase: structural analysis of an engineered change in substrate specificity. *Biochemistry* 36: 6437-6447.
4. Becker, K., et al. 1998. Enzyme inactivation through sulfhydryl oxidation by physiologic NO-carriers. *Nat. Struct. Biol.* 5: 267-271.
5. Duarte, A.I., et al. 2005. Insulin neuroprotection against oxidative stress in cortical neurons-involvement of uric acid and glutathione antioxidant defenses. *Free Radic. Biol. Med.* 39: 876-889.
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CHROMOSOMAL LOCATION

Genetic locus: GSR (human) mapping to 8p12.

PRODUCT

Glutathione reductase shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl 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 Glutathione reductase siRNA (h): sc-44843 and Glutathione reductase shRNA Plasmid (h): sc-44843-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

Glutathione reductase shRNA (h) Lentiviral Particles is recommended for the inhibition of Glutathione reductase expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl 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

Glutathione reductase (C-10): sc-133245 is recommended as a control antibody for monitoring of Glutathione reductase 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 Glutathione reductase gene expression knockdown using RT-PCR Primer: Glutathione reductase (h)-PR: sc-44843-PR (20 µl, 479 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.