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Selenoprotein R shRNA (h) Lentiviral Particles: sc-45574-V

BACKGROUND

Selenium is an essential trace element that is incorporated as selenocysteine into the primary structure of selenoproteins. Nutritional deficiency of selenium decreases selenoprotein concentrations and leads to pathologic conditions. Most of the known selenoproteins are members of the glutathione peroxidase or iodothyronine deiodinase families. Selenoprotein P (SEPP1) is a major selenoprotein that is not a member of those families. It is an extracellular glycoprotein that is present in several isoforms and is the only selenoprotein known to contain multiple selenocysteine residues. SECIS elements form stem-loop structures in the 3' untranslated regions (UTR) of eukaryotic mRNAs that encode selenoproteins. The Selenoprotein W SECIS elements contain an additional highly conserved base-paired stem that may prevent inappropriate selenocysteine incorporation at the UGA stop codons. Selenoprotein R, also designated methionine-R-sulfoxide reductase or Selenoprotein X1, is a zinc binding protein. The gene encoding for Selenoprotein R contains seleno-cysteine, which is encoded by the usual stop codon TGA at the active site. Selenoprotein R may play a role in protection against oxidative stress.

REFERENCES

- Hill, K.E., et al. 1993. Conserved nucleotide sequences in the open reading frame and 3' untranslated region of selenoprotein P mRNA. *Proc. Natl. Acad. Sci. USA* 90: 537-541.
- Chittum, H.S., et al. 1996. Multiple forms of selenoprotein P in rat plasma. *Arch. Biochem. Biophys* 325: 124-128.
- Hill, K.E., et al. 1996. Human selenoprotein P gene maps to 5q31. *Genomics* 36: 550-551.
- Kryukov, G.V. et al. 1999. New mammalian selenocysteine-containing proteins identified with an algorithm that searches for selenocysteine insertion sequence elements. *J. Biol. Chem.* 274: 33888-33897.

CHROMOSOMAL LOCATION

Genetic locus: SEPX1 (human) mapping to 16p13.3.

PRODUCT

Selenoprotein R 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 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 Selenoprotein R siRNA (h): sc-45574 and Selenoprotein R shRNA Plasmid (h): sc-45574-SH as alternate gene silencing products.

PROTOCOLS

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

APPLICATIONS

Selenoprotein R shRNA (h) Lentiviral Particles is recommended for the inhibition of Selenoprotein R 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

Selenoprotein R (E-14): sc-34846 is recommended as a control antibody for monitoring of Selenoprotein R 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Selenoprotein R gene expression knockdown using RT-PCR Primer: Selenoprotein R (h)-PR: sc-45574-PR (20 μ l). 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.

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.