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PON1 shRNA (m) Lentiviral Particles: sc-44406-V

BACKGROUND

Paroxon is an organophosphorus anticholinesterase compound, used topically in the treatment of glaucoma. It is produced *in vivo* in mammals by microsomal oxidation of the insecticide parathion. Parathion is inert until transformed to paroxon. Paroxonase or PON is an arylesterase that is capable of hydrolyzing paroxon to produce p-nitrophenol. PONs are nonspecific and their classification is based not only on substrate specificity but also on tissue distribution, inhibition properties and physicochemical characteristics such as electrophoretic mobility and molecular weight. In contrast to PON1, which is expressed mainly in the liver, PON2 is expressed in a variety of mouse tissues, including the pancreas. PON3 is associated with the high density lipoprotein fraction of serum. The genes which encode PON1-3 are physically linked and map to human chromosome 7q21.3.

REFERENCES

1. Coates, P.M., et al. 1975. A preliminary genetic interpretation of the esterase isozymes of human tissues. *Ann. Hum. Genet.* 39: 1-20.
2. Humbert, R., et al. 1993. The molecular basis of the human serum paraoxonase activity polymorphism. *Nat. Genet.* 3: 73-76.
3. Primo-Parmo, S.L., et al. 1996. The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics* 33: 498-507.
4. Mochizuki, H., et al. 1998. Human PON2 gene at 7q21.3: cloning, multiple mRNA forms, and missense polymorphisms in the coding sequence. *Gene* 213: 149-157.
5. Draganov, D.I., et al. 2000. Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. *J. Biol. Chem.* 275: 33435-33442.

CHROMOSOMAL LOCATION

Genetic locus: Pon1 (mouse) mapping to 6 A1.

PRODUCT

PON1 shRNA (m) 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 PON1 siRNA (m): sc-44406 and PON1 shRNA Plasmid (m): sc-44406-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

PON1 shRNA (m) Lentiviral Particles is recommended for the inhibition of PON1 expression in mouse 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

PON1 (N-20): sc-21146 is recommended as a control antibody for monitoring of PON1 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 PON1 gene expression knockdown using RT-PCR Primer: PON1 (m)-PR: sc-44406-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.

PROTOCOLS

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