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OGG1/2 shRNA (h) Lentiviral Particles: sc-43983-V

BACKGROUND

8-oxoguanine (8-oxoG), an oxidized form of guanine, is produced by reactive oxygen species in both DNA and nucleotide pools during normal aging. Accumulation of 8-oxoG increases the occurrence of A:T to C:G or G:C to T:A transversion mutations, because 8-oxoG forms a stable basepair with adenine as well as with cytosine. OGG1 (for 8-oxoG DNA glycosylase), also designated MMH, is a DNA repair enzyme that corrects these mutations. Inactivation of the OGG1 gene leads to a mutator phenotype, characterized by the increase in G:C to T:A transversions. The OGG1 gene encodes 8 isoforms (OGG1A-C, OGG2A-E) which result from alternative splicing of a single messenger RNA. The OGG1A splice variant is the most prevalent form and localizes to the nucleus, whereas the OGG2A splice variant is targeted to the mitochondria.

REFERENCES

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- Hayakawa, M., et al. 1993. Age-associated damage in mitochondrial DNA in human hearts. *Mol. Cell. Biochem.* 119: 95-103.
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CHROMOSOMAL LOCATION

Genetic locus: OGG1 (human) mapping to 3p25.3.

PRODUCT

OGG1/2 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 OGG1/2 siRNA (h): sc-43983 and OGG1/2 shRNA Plasmid (h): sc-43983-SH as alternate gene silencing products.

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.

APPLICATIONS

OGG1/2 shRNA (h) Lentiviral Particles is recommended for the inhibition of OGG1/2 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

OGG1/2 (G-5): sc-376935 is recommended as a control antibody for monitoring of OGG1/2 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 OGG1/2 gene expression knockdown using RT-PCR Primer: OGG1/2 (h)-PR: sc-43983-PR (20 μ l, 428 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.

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.

PROTOCOLS

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