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OGG1/2 siRNA (h): sc-43983

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 mutation, respectively, 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 GC to TA transversions. The OGG1 gene encodes eight 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 mitochondrion.

REFERENCES

- Shibutani, S., et al. 1991. Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxodG. *Nature* 349: 431-434.
- Cheng, K.C., et al. 1992. 8-hydroxyguanine, an abundant form of oxidative DNA damage, causes GT and AC substitutions. *J. Biol. Chem.* 267: 166-172.
- Ames, B.N., et al. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. USA* 90: 7915-7922.
- Hayakawa, M., et al. 1993. Age-associated damage in mitochondrial DNA in human hearts. *Mol. Cell. Biochem.* 119: 95-103.
- Nishioka, K., et al. 1999. Expression and differential intracellular localization of two major forms of human 8-oxoguanine DNA glycosylase encoded by alternatively spliced OGG1 mRNAs. *Mol. Biol. Cell* 10: 1637-1652.

CHROMOSOMAL LOCATION

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

PRODUCT

OGG1/2 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see OGG1/2 shRNA Plasmid (h): sc-43983-SH and OGG1/2 shRNA (h) Lentiviral Particles: sc-43983-V as alternate gene silencing products.

For independent verification of OGG1/2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43983A, sc-43983B and sc-43983C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

OGG1/2 siRNA (h) is recommended for the inhibition of OGG1/2 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

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° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Saha, T., et al. 2010. Transcriptional regulation of the base excision repair pathway by BRCA1. *J. Biol. Chem.* 285: 19092-19105.
- Wang, H., et al. 2014. Distinct roles of Ape1 protein, an enzyme involved in DNA repair, in high or low linear energy transfer ionizing radiation-induced cell killing. *J. Biol. Chem.* 289: 30635-30644.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

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