SANTA CRUZ BIOTECHNOLOGY, INC.

HGD siRNA (h): sc-75249

BACKGROUND

HGD (homogentisate 1,2-dioxygenase), also known as HGO (homogentisate oxygenase), is a 445 amino acid protein that belongs to the homogentisate dioxygenase family and is involved in the pathway of amino acid degradation. Expressed at high levels in kidney, colon, liver, prostate and small intestine, HGD uses iron as a cofactor to catalyze the oxygen-dependent conversion of homogentisate to 4-maleylacetoacetate, a reaction that is the fourth step in the creation of L-phenylalanine from fumarate and acetoacetic acid. Defects in the gene encoding HGD are the cause of alkaptonuria (AKU), an autosomal recessive disorder that is characterized by urine that turns dark on standing and alkalinization, black ochronotic pigmentation of cartilage and collagenous tissues and spine arthritis.

REFERENCES

1. Pollak, M.R., et al. 1993. Homozygosity mapping of the gene for alkaptonuria to chromosome 3q2. Nat. Genet. 5: 201-204.
2. Janocha, S., et al. 1994. The human gene for alkaptonuria (AKU) maps to chromosome 3q. Genomics 19: 5-8.
3. Hudecová, S., et al. 1995. Purification of the homogentisic acid oxidase from mammalian liver. Int. J. Biochem. Cell Biol. 27: 1357-1363.
4. Granadino, B., et al. 1997. The human homogentisate 1,2-dioxygenase (HGO) gene. Genomics 43: 115-122.
5. Beltrán-Valero de Bernabe, D., et al. 1998. Mutation and polymorphism analysis of the human homogentisate 1,2-dioxygenase gene in alkaptonuria patients. Am. J. Hum. Genet. 62: 776-784.
6. Ramos, S.M., et al. 1998. Molecular diagnosis of alkaptonuria mutation by analysis of homogentisate 1,2 dioxygenase mRNA from urine and blood. Am. J. Med. Genet. 78: 192-194.
7. Beltrán-Valero de Bernabe, D., et al. 1999. Analysis of alkaptonuria (AKU) mutations and polymorphisms reveals that the CCC sequence motif is a mutational hot spot in the homogentisate 1,2 dioxygenase gene (HGO). Am. J. Hum. Genet. 64: 1316-1322.
8. Titus, G.P., et al. 2000. Crystal structure of human homogentisate dioxygenase. Nat. Struct. Biol. 7: 542-546.

CHROMOSOMAL LOCATION

Genetic locus: HGD (human) mapping to 3q13.33.

PRODUCT

HGD 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HGD shRNA Plasmid (h): sc-75249-SH and HGD shRNA (h) Lentiviral Particles: sc-75249-V as alternate gene silencing products.

For independent verification of HGD (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-75249A, sc-75249B and sc-75249C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

HGD siRNA (h) is recommended for the inhibition of HGD 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 µM in 66 µl. 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

HGD (C-5): sc-376276 is recommended as a control antibody for monitoring of HGD 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 HGD gene expression knockdown using RT-PCR Primer: HGD (h)-PR: sc-75249-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.