

GCH-I siRNA (h): sc-60675

BACKGROUND

GTP cyclohydrolase I (GCH-I), a homodecamer, catalyzes the conversion of GTP into dihydroneopterin triphosphate and folate. GCH-I is the first and rate limiting enzyme in tetrahydrobiopterin (BH4) biosynthesis. BH4 is the cofactor for tyrosine hydroxylase, a rate-limiting enzyme for dopamine synthesis, and tryptophan hydroxylase, the rate-limiting enzyme for serotonin biosynthesis. Dopamine and serotonin are neurotransmitters involved in depression, which may be associated with a deficiency of BH4. Mutations in the gene encoding GCH-I cause malignant hyperphenylalaninemia, a genetic neurological disorder characterized by abnormally high levels of serum phenylalanine, and dopa-responsive dystonia (DRD), a group of movement disorders characterized by a progressive difficulty in walking which respond to L-dopa.

REFERENCES

1. Maita, N., et al. 2002. Crystal structure of the stimulatory complex of GTP cyclohydrolase I and its feedback regulatory protein GFRP. *Proc. Natl. Acad. Sci. USA* 99: 1212-1217.
2. Basset, G., et al. 2002. Folate synthesis in plants: the first step of the pterin branch is mediated by a unique bimodular GTP cyclohydrolase I. *Proc. Natl. Acad. Sci. USA* 99: 12489-12494.
3. Ishii, M., et al. 2005. Reduction of GTP cyclohydrolase I feedback regulating protein expression by hydrogen peroxide in vascular endothelial cells. *J. Pharmacol. Sci.* 97: 299-302.
4. Hagenah, J., et al. 2005. High mutation rate in dopa-responsive dystonia: detection with comprehensive GCHI screening. *Neurology* 64: 908-911.

CHROMOSOMAL LOCATION

Genetic locus: GCH1 (human) mapping to 14q22.2.

PRODUCT

GCH-I 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 GCH-I shRNA Plasmid (h): sc-60675-SH and GCH-I shRNA (h) Lentiviral Particles: sc-60675-V as alternate gene silencing products.

For independent verification of GCH-I (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-60675A, sc-60675B and sc-60675C.

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

GCH-I siRNA (h) is recommended for the inhibition of GCH-I 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

GCH-I (G-8): sc-376483 is recommended as a control antibody for monitoring of GCH-I 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 GCH-I gene expression knockdown using RT-PCR Primer: GCH-I (h)-PR: sc-60675-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Santhanam, A.V., et al. 2014. Erythropoietin increases bioavailability of tetrahydrobiopterin and protects cerebral microvasculature against oxidative stress induced by eNOS uncoupling. *J. Neurochem.* 131: 521-529.
2. Gao, Y., et al. 2016. Upregulation of AUF1 is involved in the proliferation of esophageal squamous cell carcinoma through GCH1. *Int. J. Oncol.* 49: 2001-2010.

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.