SANTA CRUZ BIOTECHNOLOGY, INC.

Ferrochelatase siRNA (h): sc-60631

BACKGROUND

Ferrochelatase, also designated Heme synthetase or protoheme ferro-lyase, is the terminal enzyme of protoheme biosynthesis that catalyzes the ferrous form of iron insertion into protoporphyrin IX. Mature ferrochelatase is a homodimeric, mitochondrial membrane-associated protein translated downstream of an N-terminal 54-amino acid transit peptide. Ferrochelatase contains two nitric oxide (NO)-sensitive clusters and coordinated 2FE-2S clusters which may potentially serve as a nitric oxide sensor. Defects in the gene encoding the Ferrochelatase enzyme, FECH, cause erythropoietic protoporhyria (EPP), which is a dominantly inherited disease of porphyrin metabolism characterized by photosensitivity and hepatobiliary disease.

REFERENCES

1. Davies, R., et al. 2005. Hepatic gene expression in protoporphyic Fech mice is associated with cholestatic injury but not a marked depletion of the heme regulatory pool. Am. J. Pathol. 166: 1041-1053.
2. Di Pierro, E., et al. 2005. A point mutation affecting an SP1 binding site in the promoter of the Ferrochelatase gene impairs gene transcription and causes erythropoietic protoporphyria. Exp. Hematol. 33: 584-591.
3. Elder, G., et al. 2005. Normal dermal Ferrochelatase activity does not protect human skin from protoporphyrin-induced photosensitivity. J. Invest. Dermatol. 125: 580.
4. Franco, R., et al. 2005. Porphyrin-substrate binding to murine Ferrochelatase: effect on the thermal stability of the enzyme. Biochem. J. 386: 599-605.
5. Najahi-Missaoui, W., et al. 2005. Production and characterization of erythropoietic protoporphyric heterodimeric Ferrochelatases. Blood 106: 1098-1104.
6. Goodwin, R.G., et al. 2005. Photosensitivity and acute liver injury in myeloproliferative disorder secondary to late-onset protoporphyria caused by deletion of a Ferrochelatase gene in hematopoietic cells. Blood 107: 60-62.
7. Ohgari, Y., et al. 2005. Ferrochelatase consisting of wild-type and mutated subunits from patients with a dominant-inherited disease, erythropoietic protoporphyria, is an active but unstable dimer. Hum. Mol. Genet. 14: 327-334.

CHROMOSOMAL LOCATION

Genetic locus: FECH (human) mapping to 18q21.31.

PRODUCT

Ferrochelatase 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 Ferrochelatase shRNA Plasmid (h): sc-60631-SH and Ferrochelatase shRNA (h) Lentiviral Particles: sc-60631-V as alternate gene silencing products.

For independent verification of Ferrochelatase (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-60631A, sc-60631B and sc-60631C.

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

Ferrochelatase siRNA (h) is recommended for the inhibition of Ferrochelatase 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

Ferrochelatase (A-3): sc-377377 is recommended as a control antibody for monitoring of Ferrochelatase 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 Ferrochelatase gene expression knockdown using RT-PCR Primer: Ferrochelatase (h)-PR: sc-60631-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Nakai, Y., et al. 2022. 5-aminolevurinic acid inhibits the proliferation of bladder cancer cells by activating heme synthesis. Oncol. Rep. 48: 186.

RESEARCH USE

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