SANTA CRUZ BIOTECHNOLOGY, INC.

ALAS-H siRNA (m): sc-44729

BACKGROUND

5-aminolevulinate synthase 1 (ALAS-H) and 2 (ALAS-E) are two isoforms of ALAS, an enzyme catalyzing the first step of the heme biosynthetic pathway in mammals. The erythroid-specific isoenzyme, ALAS-E, regulates the first step of hematopoietic cell differentation and iron metabolism in the liver. ALAS-H is a housekeeping protein which mediates synthesis of early heme in the mitochondria of most cells. Succinyl CoA associates with ALAS-E in protein conformation change and translocation of ALAS-E into the mitochondria and does not interact with ALAS-H. The ALAS-E 5'-flanking region contains binding sites for nuclear activators such as GATA-1, NF-E2 and EKLF. Since the ALAS gene maps to the X chromosome, mutation of the gene leads to the pyridoxine-re-fractory X-linked sideroblastic anemia.

REFERENCES

1. Conboy, J.G., et al. 1992. Human erythroid 5-aminolevulinate synthase. Gene structure and species-specific differences in alternative RNA splicing. J. Biol. Chem. 267: 18753-18758.
2. Kramer, M.F., et al. 2000. Transcriptional regulation of the murine erythroid-specific 5-aminolevulinate synthase gene. Gene 247: 153-166.
3. Furuyama, K., et al. 2000. Interaction between succinyl CoA synthetase and the heme-biosynthetic enzyme ALAS-E is disrupted in sideroblastic anemia. J. Clin. Invest. 105: 757-764.
4. Zhang, J., et al. 2002. Transient state kinetic investigation of 5-aminolevulinate synthase reaction mechanism. J. Biol. Chem. 277: 44660-44669.
5. Zheng, J., et al. 2008. Differential regulation of human ALAS1 mRNA and protein levels by heme and cobalt protoporphyrin. Mol. Cell. Biochem. 319: 153-161.
6. du Plessis, N., et al. 2009. Functional analysis of the 5' regulatory region of the 5-aminolevulinate synthase (ALAS1) gene in response to estrogen. Cell. Mol. Biol. 55: 20-30.
7. Degenhardt, T., et al. 2009. Peroxisome proliferator-activated receptor α controls hepatic heme biosynthesis through ALAS1. J. Mol. Biol. 388: 225-238.

CHROMOSOMAL LOCATION

Genetic locus: Alas1 (mouse) mapping to 9 F1.

PRODUCT

ALAS-H siRNA (m) 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 ALAS-H shRNA Plasmid (m): sc-44729-SH and ALAS-H shRNA (m) Lentiviral Particles: sc-44729-V as alternate gene silencing products.

For independent verification of ALAS-H (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44729A, sc-44729B and sc-44729C.

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

ALAS-H siRNA (m) is recommended for the inhibition of ALAS-H expression in mouse 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

ALAS-H (F-5): sc-137093 is recommended as a control antibody for monitoring of ALAS-H 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 ALAS-H gene expression knockdown using RT-PCR Primer: ALAS-H (m)-PR: sc-44729-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.