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ALAS-H siRNA (h): sc-44728

BACKGROUND

5-aminolevulinic acid 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 differentiation 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-refractory X-linked sideroblastic anemia.

REFERENCES

1. Conboy, J.G., et al. 1992. Human erythroid 5-aminolevulinic acid 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-aminolevulinic acid 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-aminolevulinic acid synthase reaction mechanism. *J. Biol. Chem.* 277: 44660-44669.

CHROMOSOMAL LOCATION

Genetic locus: ALAS1 (human) mapping to 3p21.2.

PRODUCT

ALAS-H 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 ALAS-H shRNA Plasmid (h): sc-44728-SH and ALAS-H shRNA (h) Lentiviral Particles: sc-44728-V as alternate gene silencing products.

For independent verification of ALAS-H (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-44728A, sc-44728B and sc-44728C.

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

ALAS-H siRNA (h) is recommended for the inhibition of ALAS-H expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29527 (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

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-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, TBS Blotto A Blocking Reagent: sc-2333 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 ALAS-H gene expression knockdown using RT-PCR Primer: ALAS-H (h)-PR: sc-44728-PR (20 μ l, 511 bp). 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.