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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
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SANTA CRUZ BIOTECHNOLOGY, INC.

ALAS-E siRNA (h): sc-44726

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-refractory X-linked sideroblastic anemia.

REFERENCES

1. Conboy, J.G., Cox, T.C., Bottomley, S.S., Bawden, M.J. and May, B.K. 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., Gunaratne, P. and Ferreira, G.C. 2000. Transcriptional regulation of the murine erythroid-specific 5-aminolevulinate synthase gene. Gene 247: 153-166.
3. Furuyama, K. and Sassa, S. 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. and Ferreira, G.C. 2002. Transient state kinetic investigation of 5-aminolevulinate synthase reaction mechanism. J. Biol. Chem. 277: 44660-44669.

CHROMOSOMAL LOCATION

Genetic locus: ALAS2 (human) mapping to Xp11.21.

PRODUCT

ALAS-E 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 ALAS-E shRNA Plasmid (h): sc-44726-SH and ALAS-E shRNA (h) Lentiviral Particles: sc-44726-V as alternate gene silencing products.

For independent verification of ALAS-E (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-44726A, sc-44726B and sc-44726C.

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.

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-E siRNA (h) is recommended for the inhibition of ALAS-E 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

ALAS-E (D-4): sc-166739 is recommended as a control antibody for monitoring of ALAS-E 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-E gene expression knockdown using RT-PCR Primer: ALAS-E (h)-PR: sc-44726-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.