SANTA CRUZ BIOTECHNOLOGY, INC.

AUH siRNA (h): sc-72593

BACKGROUND

AUH (AU-binding protein/enoyl-CoA hydratase), also known as mitochondrial methylglutaconyl-CoA hydratase, is a 339 amino acid member of the enoyl-CoA hydratase/isomerase family. AUH is involved in the amino acid degradation pathway by catalyzing the conversion of 3-methylglutaconyl-CoA to 3-hydroxy-3-methylglutaryl-CoA and water. Localized to the mitochondria, AUH has been found to have very low enoyl-CoA hydratase activity. AUH is expressed as two isoforms produced by alternative splicing and forms a homohexamer. Defects in AUH result in 3-methylglutaconic aciduria type 1 (MGA1), an inborn error of leucine metabolism. MGA1 has a varied clinical phenotype, including coma, severe psychomotor retardation, delayed speech development, failure to thrive, metabolic acidosis and dystonia.

REFERENCES

1. Nakagawa, J., et al. 1995. AUH, a gene encoding an AU-specific RNA binding protein with intrinsic enoyl-CoA hydratase activity. Proc. Natl. Acad. Sci. USA 92: 2051-2055.
2. Nakagawa, J. and Moroni, C. 1997. A 20-amino-acid autonomous RNA-binding domain contained in an enoyl-CoA hydratase. Eur. J. Biochem. 244: 890-899.
3. Brennan, L.E., et al. 1999. Characterisation and mitochondrial localisation of AUH, an AU-specific RNA-binding enoyl-CoA hydratase. Gene 228: 85-91.
4. Kurimoto, K., et al. 2001. Crystal structure of human AUH protein, a single-stranded RNA binding homolog of enoyl-CoA hydratase. Structure 9: 1253-1263.
5. IJlst, L., et al. 2002. 3-Methylglutaconic aciduria type I is caused by mutations in AUH. Am. J. Hum. Genet. 71: 1463-1466.
6. Ly, T.B., et al. 2003. Mutations in the AUH gene cause 3-methylglutaconic aciduria type I. Hum. Mutat. 21: 401-407.
7. IIlsinger, S., et al. 2004. 3-methylglutaconic aciduria type I in a boy with fever-associated seizures. Pediatr. Neurol. 30: 213-215.

CHROMOSOMAL LOCATION

Genetic locus: AUH (human) mapping to 9q22.31.

PRODUCT

AUH 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 AUH shRNA Plasmid (h): sc-72593-SH and AUH shRNA (h) Lentiviral Particles: sc-72593-V as alternate gene silencing products.

For independent verification of AUH (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-72593A, sc-72593B and sc-72593C.

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

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

AUH (B-8): sc-518216 is recommended as a control antibody for monitoring of AUH 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 AUH gene expression knockdown using RT-PCR Primer: AUH (h)-PR: sc-72593-PR (20 µl, 427 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.