SANTA CRUZ BIOTECHNOLOGY, INC.

AASS siRNA (m): sc-140738

BACKGROUND

α-aminoadipic semialdehyde synthase (AASS), also designated lysine ketoglutarate reductase (LKR) or saccharopine dehydrogenase (SDH), is a 926 amino acid protein that exists as a homodimer in the mitochondria. AASS acts as a bifunctional enzyme containing the lysine α-ketoglutarate reductase (LKR) and saccharopine dehydrogenase activities that catalyzes the first two steps in lysine degradation. It is widely expressed with highest expression in liver and transcription of the AASS gene is induced upon starvation. Mutations in the gene encoding AASS result in various forms familial hyperlysinemias (FH), autosomal recessive disorders characterized by hyperlysinemia, lysinuria, and variable saccharopinuria. However, no adverse mental or physical effects have been found in patients with hyperlysinemia.

REFERENCES

1. Dancis, J., et al. 1976. Multiple enzyme defects in familial hyperlysinemia. Pediatr. Res. 10: 686-691.
2. Markovitz, P.J., et al. 1984. Familial hyperlysinemias. Purification and characterization of the bifunctional aminoadipic semialdehyde synthase with lysine-ketoglutarate reductase and saccharopine dehydrogenase activities. J. Biol. Chem. 259: 11643-11646.
3. Oyanagi, K., et al. 1998. Familial hyperlysinemia(α-aminoadipic semialdehyde synthase defect). Ryoikibetsu Shokogun Shirizu 18: 188-190.
4. Sacksteder, K.A., et al. 2000. Identification of the α-aminoadipic semialdehyde synthase gene, which is defective in familial hyperlysinemia. Am. J. Hum. Genet. 66: 1736-1743.
5. Praphanphoj, V., et al. 2001. Identification of the α-aminoadipic semialdehyde dehydrogenase-phosphopantetheinyl transferase gene, the human ortholog of the yeast LYS5 gene. Mol. Genet. Metab. 72: 336-342.
6. Cleveland, B.M., et al. 2008. α-aminoadipate δ-semialdehyde synthase mRNA knockdown reduces the lysine requirement of a mouse hepatic cell line. J. Nutr. 138: 2143-2147.
7. Tonin, A.M., et al. 2009. Inhibition of creatine kinase activity by lysine in rat cerebral cortex. Metab. Brain Dis. 24: 349-360.

CHROMOSOMAL LOCATION

Genetic locus: Aass (mouse) mapping to 6 A3.1.

PRODUCT

AASS 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. Suita-ble for 50-100 transfections. Also see AASS shRNA Plasmid (m): sc-140738-SH and AASS shRNA (m) Lentiviral Particles: sc-140738-V as alternate gene silencing products.

For independent verification of AASS (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-140738A, sc-140738B and sc-140738C.

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

AASS siRNA (m) is recommended for the inhibition of AASS 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

AASS (H-5): sc-390511 is recommended as a control antibody for monitoring of AASS 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 AASS gene expression knockdown using RT-PCR Primer: AASS (m)-PR: sc-140738-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.