

Aminoacylase-1 siRNA (h): sc-61966

BACKGROUND

Aminoacylase-1, also designated N-acyl-L-amino-acid amidohydrolase or ACY-1, is a member of the largest metallopeptidase family called M20A. Aminoacylase-1 is a zinc-binding homodimeric enzyme expressed in kidney, brain, placenta and spleen. It is the most abundant of the aminoacylases. Aminoacylase-1 localizes to the cytoplasm and catalyzes the hydrolysis of N-acylated or N-acetylated amino acids. In addition, Aminoacylase-1 is a sphingosine kinase 1 (SphK1)-interacting protein and may also play a role in regulating responses of the cell to oxidative stress. The gene encoding Aminoacylase-1 is evolutionarily conserved in fish, frog, mouse, rat and human. Deficiency in the Aminoacylase-1 protein may result in defects of brain metabolism and function.

REFERENCES

1. Cook, R.M., et al. 1993. Human aminoacylase-1. Cloning, sequence, and expression analysis of a chromosome 3p21 gene inactivated in small cell lung cancer. *J. Biol. Chem.* 268: 17010-17017.
2. Lindner, H., et al. 2001. Mutational analysis of two PWW sequence motifs in human aminoacylase 1. *Biol. Chem.* 381: 1055-1061.
3. Lindner, H.A., et al. 2003. Essential roles of zinc ligation and enzyme dimerization for catalysis in the aminoacylase-1/M20 family. *J. Biol. Chem.* 278: 44496-44504.
4. Maceyka, M., et al. 2004. Aminoacylase 1 is a sphingosine kinase 1-interacting protein. *FEBS Lett.* 568: 30-34.
5. Lindner, H.A., et al. 2005. Roles of dimerization domain residues in binding and catalysis by aminoacylase-1. *Biochemistry* 44: 15645-15651.
6. Liu, Z., et al. 2006. Probing the catalytic center of porcine aminoacylase 1 by site-directed mutagenesis, homology modeling and substrate docking. *J. Biochem.* 139: 421-430.
7. Sass, J.O., et al. 2006. Mutations in ACY, the gene encoding aminoacylase 1, cause a novel inborn error of metabolism. *Am. J. Hum. Genet.* 78: 401-409.
8. Engelke, U.F., et al. 2007. NMR spectroscopy of aminoacylase 1 deficiency, a novel inborn error of metabolism. *NMR Biomed.* 21: 138-147.

CHROMOSOMAL LOCATION

Genetic locus: ACY1 (human) mapping to 3p21.2.

PRODUCT

Aminoacylase-1 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 Aminoacylase-1 shRNA Plasmid (h): sc-61966-SH and Aminoacylase-1 shRNA (h) Lentiviral Particles: sc-61966-V as alternate gene silencing products.

For independent verification of Aminoacylase-1 (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-61966A, sc-61966B and sc-61966C.

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

Aminoacylase-1 siRNA (h) is recommended for the inhibition of Aminoacylase-1 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 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

Aminoacylase-1 (B-1): sc-271019 is recommended as a control antibody for monitoring of Aminoacylase-1 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, UltraCruz[®] Blocking Reagent: sc-516214 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 Aminoacylase-1 gene expression knockdown using RT-PCR Primer: Aminoacylase-1 (h)-PR: sc-61966-PR (20 μ l). 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.