SANTA CRUZ BIOTECHNOLOGY, INC.

DAAO siRNA (h): sc-105269

BACKGROUND

D-amino acid oxidase (DAAO) metabolizes exogenous D-amino acids that accumulate during aging, and may modulate the level of D-serine in the brain, acting as a detoxifying agent. DAAO is specific for the D-configuration of amino acids and exhibits a preference for those with small, hydrophobic side chains and polar, aromatic and basic functional groups. Single base-pair substitutions at certain amino acid residues of the DAAO enzyme result in the loss of DAO activity in mice. Mutation of the enzyme at residue Y238, one of the few conserved residues in the active site of DAAO, leads to a significantly slower rate of product release relative to the wild-type enzyme, indicating the importance of this residue in controlling access to the active site. In addition, the other conserved residues in the active site of DAAO do not play a role in acid-base catalysis but rather are involved in substrate interactions, which along with the mode of substrate orientation into the active site, suggest a hybrid transfer type of mechanism for catalysis.

REFERENCES

1. Sasaki, M., Konno, R., Nishio, M., Niwa, A., Yasumura, Y. and Enami, J. 1992. A single-base-pair substitution abolishes D-amino-acid oxidase activity in the mouse. Biochim. Biophys. Acta 1139: 315-318.
2. Mujawar, S.K. 1999. D-amino acid oxidase: its potential in the production of 7-aminocephalosporanic acid. Hindustan Antibiot. Bull. 41: 1-14.
3. Pilone, M.S. 2000. D-amino acid oxidase: new findings. Cell. Mol. Life Sci. 57: 1732-1747.
4. Boselli, A., Sacchi, S., Job, V., Pilone, M.S. and Pollegioni, L. 2002. Role of tyrosine 238 in the active site of Rhodotorula gracilis D-amino acid oxidase. A site-directed mutagenesis study. Eur. J. Biochem. 269: 4762-4771.
5. Caldinelli, L., Iametti, S., Barbiroli, A., Bonomi, F., Piubelli, L., Ferranti, P., Picariello, G., Pilone, M.S. and Pollegioni, L. 2004. Unfolding intermediate in the peroxisomal flavoprotein D-amino acid oxidase. J. Biol. Chem. 279: 28426-28434.
6. SWISS-PROT/TrEMBL (P18894). World Wide Web URL: http://www.expasy.ch/sprot/sprot-top.html

CHROMOSOMAL LOCATION

Genetic locus: DAO (human) mapping to 12q24.11.

PRODUCT

DAAO 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 DAAO shRNA Plasmid (h): sc-105269-SH and DAAO shRNA (h) Lentiviral Particles: sc-105269-V as alternate gene silencing products.

For independent verification of DAAO (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-105269A, sc-105269B and sc-105269C.

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

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

DAAO (B-3): sc-398757 is recommended as a control antibody for monitoring of DAAO 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 DAAO gene expression knockdown using RT-PCR Primer: DAAO (h)-PR: sc-105269-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.