SANTA CRUZ BIOTECHNOLOGY, INC.

DDAH I siRNA (m): sc-142914

BACKGROUND

DDAH, a dimethylarginine dimethylaminohydrolase, hydrolyzes dimethyl arginine (ADMA) and monomethyl arginine (MMA), both inhibitors of nitric oxide synthases, and may be involved in *in vivo* modulation of nitric oxide production. Impairment of DDAH causes ADMA accumulation and a reduction in cGMP generation. DDAH II, the predominant DDAH isoform in endothelial cells, facilitates the induction of nitric oxide synthesis by all-*trans*-Retinoic acid (atRA). DDAH proteins are highly expressed in colon, kidney, stomach and liver tissues.

REFERENCES

1. Nakagomi, S., et al. 1999. Dimethylarginine dimethylaminohydrolase (DDAH) as a nerve-injury-associated molecule: mRNA localization in the rat brain and its coincident upregulation with neuronal NO synthase (nNOS) in axotomized motoneurons. Eur. J. Neurosci. 11: 2160-2166.
2. Knipp, M., et al. 2001. Structural and functional characterization of the Zn(II) site in dimethylargininase-1 (DDAH I) from bovine brain. Zn(II) release activates DDAH I. J. Biol. Chem. 276: 40449-40456.
3. Leiper, J., et al. 2002. S-nitrosylation of dimethylarginine dimethylaminohydrolase regulates enzyme activity: further interactions betwen nitric oxide synthase and dimethylarginine dimethylaminohydrolase. Proc. Natl. Acad. Sci. USA 99: 13527-13532.
4. Lin, K.Y., et al. 2002. Impaired nitric oxide synthase pathway in diabetes mellitus: role of asymmetric dimethylarginine and dimethylaminohydrolase. Circulation 106: 987-992.
5. Achan, V., et al. 2002. All-*trans*-retinoic acid increases nitric oxide synthesis by endothelial cells: a role for the induction of dimethylaminohydrolase. Circ. Res. 90: 764-769.
6. Knipp, M., et al. 2003. Zn(II)-free dimethylargininase I (DDAH I) is inhibited upon specific Cys-S-nitrosylation. J. Biol. Chem. 278: 3410-3416.
7. Swiss-Prot/TrEMBL (O94760). World Wide Web URL: http://www.expasy.ch/sprot/sprot-top.html

CHROMOSOMAL LOCATION

Genetic locus: Ddah1 (mouse) mapping to 3 H2.

PRODUCT

DDAH I siRNA (m) is a pool of 2 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 DDAH I shRNA Plasmid (m): sc-142914-SH and DDAH I shRNA (m) Lentiviral Particles: sc-142914-V as alternate gene silencing products.

For independent verification of DDAH I (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-142914A and sc-142914B.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

DDAH I siRNA (m) is recommended for the inhibition of DDAH I 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

DDAH I (D-6): sc-514841 is recommended as a control antibody for monitoring of DDAH I 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 DDAH I gene expression knockdown using RT-PCR Primer: DDAH I (m)-PR: sc-142914-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.