

DDAH II siRNA (h): sc-40474

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 between 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.

CHROMOSOMAL LOCATION

Genetic locus: DDAH2 (human) mapping to 6p21.33.

PRODUCT

DDAH II 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 DDAH II shRNA Plasmid (h): sc-40474-SH and DDAH II shRNA (h) Lentiviral Particles: sc-40474-V as alternate gene silencing products.

For independent verification of DDAH II (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-40474A, sc-40474B and sc-40474C.

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

DDAH II siRNA (h) is recommended for the inhibition of DDAH II 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

DDAH II (3E3): sc-293229 is recommended as a control antibody for monitoring of DDAH II 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 DDAH II gene expression knockdown using RT-PCR Primer: DDAH II (h)-PR: sc-40474-PR (20 μ l, 488 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Hu, X., et al. 2011. Dimethylarginine dimethylaminohydrolase-1 is the critical enzyme for degrading the cardiovascular risk factor asymmetrical dimethylarginine. *Arterioscler. Thromb. Vasc. Biol.* 31: 1540-1546.
2. Jiang, N., et al. 2016. N-methylnicotinamide protects against endothelial dysfunction and attenuates atherogenesis in apolipoprotein E-deficient mice. *Mol. Nutr. Food Res.* 60: 1625-1636.
3. Lin, H.H., et al. 2019. DDAH-2 alleviates contrast medium iopromide-induced acute kidney injury through nitric oxide synthase. *Clin. Sci.* 133: 2361-2378.

RESEARCH USE

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