

DHODH siRNA (m): sc-77142

BACKGROUND

DHODH (dihydroorotate dehydrogenase), also known as DHodehase, is a 395 amino acid mitochondrial protein located on the outer surface of the inner mitochondrial membrane. It catalyzes the fourth enzymatic step in *de novo* pyrimidine biosynthesis. *De novo* pyrimidine synthesis is a critical metabolic pathway for nucleic acid synthesis and is a target for various cancer chemotherapy agents. Additionally, DHODH is functionally connected to the respiratory chain, delivering electrons to ubiquinone. DHODH contains a bipartite signal at the N-terminus that regulates passage into the mitochondrial inner membrane. The inhibition of COX (cytochrome c oxidase) by nitric oxide (NO) indirectly inhibits DHODH activity. The inhibition of DHODH has an immunosuppressive and an antiproliferative effect on diseases such as rheumatoid arthritis.

REFERENCES

1. Barnes, T., et al. 1993. Regional mapping of the gene encoding dihydroorotate dehydrogenase, an enzyme involved in UMP synthesis, electron transport, and superoxide generation, to human chromosome region 16q22. *Somat. Cell Mol. Genet.* 19: 405-411.
2. Copeland, R.A., et al. 1995. Recombinant human dihydroorotate dehydrogenase: expression, purification, and characterization of a catalytically functional truncated enzyme. *Arch. Biochem. Biophys.* 323: 79-86.
3. Knecht, W., et al. 1996. Functional expression of a fragment of human dihydroorotate dehydrogenase by means of the baculovirus expression vector system, and kinetic investigation of the purified recombinant enzyme. *Eur. J. Biochem.* 240: 292-301.
4. Beuneu, C., et al. 2000. Indirect inhibition of mitochondrial dihydroorotate dehydrogenase activity by nitric oxide. *Free Radic. Biol. Med.* 28: 1206-1213.
5. Dietz, C., et al. 2000. Immunocytochemical detection of mitochondrial dihydroorotate dehydrogenase in human spermatozoa. *Int. J. Androl.* 23: 294-299.
6. Rawls, J., et al. 2000. Requirements for the mitochondrial import and localization of dihydroorotate dehydrogenase. *Eur. J. Biochem.* 267: 2079-2087.

CHROMOSOMAL LOCATION

Genetic locus: Dhodh (mouse) mapping to 8 D3.

PRODUCT

DHODH 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DHODH shRNA Plasmid (m): sc-77142-SH and DHODH shRNA (m) Lentiviral Particles: sc-77142-V as alternate gene silencing products.

For independent verification of DHODH (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-77142A, sc-77142B and sc-77142C.

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

DHODH siRNA (m) is recommended for the inhibition of DHODH 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 μ M in 60 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

DHODH (E-8): sc-166348 is recommended as a control antibody for monitoring of DHODH 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 DHODH gene expression knockdown using RT-PCR Primer: DHODH (m)-PR: sc-77142-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.