SANTA CRUZ BIOTECHNOLOGY, INC.

DLD siRNA (m): sc-62219

BACKGROUND

DLD (dihydrolipoyl dehyrogenase or dihydrolipoamide dehydrogenase), also known as GCSL (glycine cleavage system L protein), PHE3, DLDH or LAD, is a member of the class I pyridine nucleotide-disulfide oxidoreductase family. DLD is a flavin-dependent oxidoreductase and functions as a component of the α-keto acid dehydrogenase, the pyruvate dehydrogenase, the α-ketoglutarate dehydrogenase, the branched-chain α-keto acid dehydrogenase and as the L protein in the mitochondrial glycine cleavage system. DLD localizes to the mitochondrial matrix and exists as a monomer, homodimer or tetramer that is required for energy metabolism in all eukaryotes. More specifically, DLD generates NADH and lipoic acid from dihydrolipoic acid and NAD^+. The DLD homodimer catalyzes the opposite reaction. Mutations in the gene encoding DLD can result in MSUD (maple syrup urine disease) and congenital infantile lactic acidosis.

REFERENCES

1. Brown, A.M., et al. 2004. Association of the dihydrolipoamide dehydrogenase gene with Alzheimer's disease in an Ashkenazi Jewish population. Am. J. Med. Genet. B Neuropsychiatr. Genet. 131: 60-66.
2. Starkov, A.A., et al. 2004. Mitochondrial α-ketoglutarate dehydrogenase complex generates reactive oxygen species. J. Neurosci. 24: 7779-7788.
3. Nishimoto, E., et al. 2006. Thermal unfolding process of dihydrolipoamide dehydrogenase studied by fluorescence spectroscopy. J. Biochem. 140: 349-357.
4. Cameron, J.M., et al. 2006. Novel mutations in dihydrolipoamide dehydrogenase deficiency in two cousins with borderline-normal PDH complex activity. Am. J. Med. Genet. A 140: 1542-1552.
5. Smolle, M., et al. 2006. A new level of architectural complexity in the human pyruvate dehydrogenase complex. J. Biol. Chem. 281: 19772-19780.
6. Kim, H. 2006. Activity of human dihydrolipoamide dehydrogenase is largely reduced by mutation at isoleucine-51 to alanine. J. Biochem. Mol. Biol. 39: 223-227.

CHROMOSOMAL LOCATION

Genetic locus: Dld (mouse) mapping to 12 A3.

PRODUCT

DLD 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DLD shRNA Plasmid (m): sc-62219-SH and DLD shRNA (m) Lentiviral Particles: sc-62219-V as alternate gene silencing products.

For independent verification of DLD (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of ly-ophilized siRNA. These include: sc-62219A, sc-62219B and sc-62219C.

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

DLD siRNA (m) is recommended for the inhibition of DLD 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

DLD (G-2): sc-365977 is recommended as a control antibody for monitoring of DLD 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 DLD gene expression knockdown using RT-PCR Primer: DLD (m)-PR: sc-62219-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.