

PDH-E1 α siRNA (m): sc-77407

BACKGROUND

The pyruvate dehydrogenase (PDH) complex is a nuclear-encoded mitochondrial matrix enzyme complex that functions as the primary link between glycolysis and the tricarboxylic acid (TCA) cycle by catalyzing the irreversible conversion of pyruvate into acetyl-CoA. The E1 enzyme of the PDH complex is made up of a heterotetramer of two α and two β subunits. The E1 α subunit (PDH-E1 α) contains the E1 active site and plays a key role in the function of the PDH complex. The PDH complex is regulated by phosphorylation and dephosphorylation of PDH-E1 α . The gene encoding for PDH-E1 α maps to chromosome Xp22.12, and a 20-bp deletion in the last exon of this gene is sufficient to cause PDH deficiency, which causes a broad range of symptoms including the development of seizures, mental retardation and spasticity, as well as intermittent episodes of lactic acidosis associated with cerebellar ataxia.

REFERENCES

1. Sermon, K., et al. 1990. Characterisation of a cDNA for porcine PDH-E1 α and comparison with the human cDNA. *Nucleic Acids Res.* 18: 4925.
2. Chun, K., et al. 1991. Pyruvate dehydrogenase deficiency due to a 20-bp deletion in exon II of the pyruvate dehydrogenase (PDH) E1 α gene. *Am. J. Hum. Genet.* 49: 414-420.
3. Chun, K., et al. 1993. Mutations in the X-linked E1 α subunit of pyruvate dehydrogenase leading to deficiency of the pyruvate dehydrogenase complex. *Hum. Mol. Genet.* 2: 449-454.
4. Hansen, L.L., et al. 1994. Pyruvate dehydrogenase deficiency caused by a 33 base pair duplication in the PDH-E1 α subunit. *Hum. Mol. Genet.* 3: 1021-1022.
5. Brown, G.K., et al. 1995. Pyruvate dehydrogenase deficiency. *J. Med. Genet.* 31: 875-879.
6. Otero, L.J., et al. 1996. Association of cerebral dysgenesis and lactic acidemia with x-linked PDH-E1 α subunit mutations in females. *Pediatr. Neurol.* 13: 327-332.

CHROMOSOMAL LOCATION

Genetic locus: Pdha1 (mouse) mapping to X F4.

PRODUCT

PDH-E1 α 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 PDH-E1 α shRNA Plasmid (m): sc-77407-SH and PDH-E1 α shRNA (m) Lentiviral Particles: sc-77407-V as alternate gene silencing products.

For independent verification of PDH-E1 α (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-77407A, sc-77407B and sc-77407C.

PROTOCOLS

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

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

PDH-E1 α siRNA (m) is recommended for the inhibition of PDH-E1 α 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 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

PDH-E1 α (D-6): sc-377092 is recommended as a control antibody for monitoring of PDH-E1 α 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 PDH-E1 α gene expression knockdown using RT-PCR Primer: PDH-E1 α (m)-PR: sc-77407-PR (20 μ l, 474 bp). 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.