SANTA CRUZ BIOTECHNOLOGY, INC.

PDH-E1α siRNA (h): sc-91064

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 E1α subunit (PDH-E1α) 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-E1α. The gene encoding for PDH-E1α 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-E1α 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) E1α gene. Am. J. Hum. Genet. 49: 414-420.
3. Chun, K., et al. 1993. Mutations in the X-linked E1α 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-E1α subunit. Hum. Mol. Genet. 3: 1021-1022.

CHROMOSOMAL LOCATION

Genetic locus: PDHA1 (human) mapping to Xp22.12.

PRODUCT

PDH-E1α 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PDH-E1α shRNA Plasmid (h): sc-91064-SH and PDH-E1α shRNA (h) Lentiviral Particles: sc-91064-V as alternate gene silencing products.

For independent verification of PDH-E1α (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-91064A, sc-91064B and sc-91064C.

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

PDH-E1α siRNA (h) is recommended for the inhibition of PDH-E1α 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 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

PDH-E1α (D-6): sc-377092 is recommended as a control antibody for monitoring of PDH-E1α 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 PDH-E1α gene expression knockdown using RT-PCR Primer: PDH-E1α (h)-PR: sc-91064-PR (20 µl, 594 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Islam, R., et al. 2019. Insulin induces phosphorylation of pyruvate dehydrogenase through RhoA activation pathway in Hep G2 cells. FASEB J. 33: 2072-2083.
2. Prasad, P., et al. 2021. Glutamine deficiency promotes stemness and chemoresistance in tumor cells through DRP1-induced mitochondrial fragmentation. Cell. Mol. Life Sci. 78: 4821-4845.
3. Hossain, A.J., et al. 2022. Pyruvate dehydrogenase A1 phosphorylated by Insulin associates with pyruvate kinase M2 and induces LINC00273 through histone acetylation. Biomedicines 10: 1256.

RESEARCH USE

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