

HPPD siRNA (m): sc-75298

BACKGROUND

HPPD (4-hydroxyphenylpyruvate dioxygenase), also known as PPD, GLOD3 or HPD, is a 393 amino acid protein that belongs to the 4HPPD family and is involved in amino acid degradation. Existing as a homodimer, HPPD uses zinc as a cofactor to catalyze the third step in the conversion of L-phenylalanine to fumarate and acetoacetic acid. Defects in the gene encoding HPPD are the cause of tyrosinemia type 3 (TYRO3) and hawkinsinuria (HAWK), both of which are inborn errors of metabolism that are associated with a variety of symptoms, including mental retardation and seizures (associated with TYRO3) and hair and urine abnormalities (associated with HAWK). The gene encoding HPPD maps to human chromosome 12, which encodes over 1,100 genes and comprises approximately 4.5% of the human genome.

REFERENCES

1. Rüetschi, U., et al. 1993. Human 4-hydroxyphenylpyruvate dioxygenase. Primary structure and chromosomal localization of the gene. *Eur. J. Biochem.* 213: 1081-1089.
2. Awata, H., et al. 1994. Structure of the human 4-hydroxyphenylpyruvic acid dioxygenase gene (HPD). *Genomics* 23: 534-539.
3. Stenman, G., et al. 1995. Regional assignment of the human 4-hydroxyphenylpyruvate dioxygenase gene (HPD) to 12q24→qter by fluorescence *in situ* hybridization. *Cytogenet. Cell Genet.* 71: 374-376.
4. Rüetschi, U., et al. 1997. Human 4-hydroxyphenylpyruvate dioxygenase gene (HPD). *Genomics* 44: 292-299.
5. Rüetschi, U., et al. 2000. Mutations in the 4-hydroxyphenylpyruvate dioxygenase gene (HPD) in patients with tyrosinemia type III. *Hum. Genet.* 106: 654-662.
6. Tomoeda, K., et al. 2000. Mutations in the 4-hydroxyphenylpyruvic acid dioxygenase gene are responsible for tyrosinemia type III and hawkinsinuria. *Mol. Genet. Metab.* 71: 506-510.
7. Item, C.B., et al. 2007. Manifestation of hawkinsinuria in a patient compound heterozygous for hawkinsinuria and tyrosinemia III. *Mol. Genet. Metab.* 91: 379-383.

CHROMOSOMAL LOCATION

Genetic locus: Hpd (mouse) mapping to 5 F.

PRODUCT

HPPD 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 HPPD shRNA Plasmid (m): sc-75298-SH and HPPD shRNA (m) Lentiviral Particles: sc-75298-V as alternate gene silencing products.

For independent verification of HPPD (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-75298A, sc-75298B and sc-75298C.

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

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

HPPD (B-11): sc-390279 is recommended as a control antibody for monitoring of HPPD 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 HPPD gene expression knockdown using RT-PCR Primer: HPPD (m)-PR: sc-75298-PR (20 μ l, 598 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.

PROTOCOLS

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