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PEPD siRNA (m): sc-152165

BACKGROUND

PEPD (peptidase D), also referred to as prolidase, is a cytosolic dipeptidase that belongs to the peptidase M24B family. PEPD hydrolyzes di- and tripeptides with proline or hydroxyproline at the C-terminus. PEPD functions as a homodimer and may play an important role in collagen metabolism as well as in the recycling of proline in various cells and tissues. Defects in the gene encoding PEPD are the primary cause of prolidase deficiency in humans. Prolidase deficiency is an autosomal recessive disorder associated with iminodipeptiduria and is characterized by skin ulcers, mental retardation, recurrent infections and A-typical facies. Mutations in the gene encoding PEPD may also be the cause of systemic lupus erythematosus and necrosis-like cell death in fibroblasts. Additionally, there is thought to be a tight linkage between the polymorphisms of prolidase and the myotonic dystrophy trait.

REFERENCES

1. Leoni, A., et al. 1987. Prolidase deficiency in two siblings with chronic leg ulcerations. Clinical, biochemical, and morphologic aspects. Arch. Dermatol. 123: 493-499.
2. Boright, A.P., et al. 1989. Prolidase deficiency: biochemical classification of alleles. Am. J. Hum. Genet. 44: 731-740.
3. Tanoue, A., et al. 1990. Structural organization of the gene for human prolidase (peptidase D) and demonstration of a partial gene deletion in a patient with prolidase deficiency. J. Biol. Chem. 265: 11306-11311.
4. Endo, F. and Matsuda, I. 1991. Molecular basis of prolidase (peptidase D) deficiency. Mol. Biol. Med. 8: 117-127.
5. Henrich, B., et al. 1992. The promoter region of the *Escherichia coli* pepD gene: deletion analysis and control by phosphate concentration. Mol. Gen. Genet. 232: 117-125.
6. Ledoux, P., et al. 1994. Four novel PEPD alleles causing prolidase deficiency. Am. J. Hum. Genet. 54: 1014-1021.
7. Kikuchi, S., et al. 2000. A novel nonsense mutation of the PEPD gene in a Japanese patient with prolidase deficiency. J. Hum. Genet. 45: 102-104.
8. Forlino, A., et al. 2002. Mutation analysis of five new patients affected by prolidase deficiency: the lack of enzyme activity causes necrosis-like cell death in cultured fibroblasts. Hum. Genet. 111: 314-322.

CHROMOSOMAL LOCATION

Genetic locus: PEPD (mouse) mapping to 7 B1.

PRODUCT

PEPD 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 PEPD shRNA Plasmid (m): sc-152165-SH and PEPD shRNA (m) Lentiviral Particles: sc-152165-V as alternate gene silencing products.

For independent verification of PEPD (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-152165A, sc-152165B and sc-152165C.

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

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

PEPD (A-3): sc-390042 is recommended as a control antibody for monitoring of PEPD 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 MarkerTM 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 PEPD gene expression knockdown using RT-PCR Primer: PEPD (m)-PR: sc-152165-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.