

PAH siRNA (h): sc-41528

BACKGROUND

The PAH gene encodes the enzyme phenylalanine hydroxylase (PAH), which converts phenylalanine to tyrosine and is the rate-limiting enzyme in phenylalanine catabolism. Mammalian PAH is a soluble, homotetrameric protein which is abundantly expressed in human liver. Deficiency of PAH activity results in the autosomal recessive disorder phenylketonuria (PKU), which is characterized by mental retardation unless a low phenylalanine diet is introduced early in life. The PAH gene, which maps to human chromosome 12q23.2, contains all the genetic information necessary to code for functional PAH, demonstrating that a single gene is involved in the classic disease phenotype. Numerous mutations can impair the PAH gene, which result in decreased enzyme activity and give rise to varying degrees of PKU. Multiple isozymes of PAH have been reported to exist, but these are most likely allelic variants of PAH that produce protein subunits with slightly different charge and electrophoretic migration.

REFERENCES

1. Hopkinson, D.A., et al. 1976. The distributions of subunit numbers and subunit sizes of enzymes: a study of the products of 100 human gene loci. *Ann. Hum. Genet.* 39: 383-411.
2. Ledley, F.D., et al. 1985. Gene transfer and expression of human phenylalanine hydroxylase. *Science* 228: 77-79.
3. Chestkov, V.V. and Laptev, A.V. 1988. Immunochemical detection and characteristics of the subunit composition of phenylalanine hydroxylase in the brain of man. *Biull. Eksp. Biol. Med.* 106: 30-34.
4. Wang, T., et al. 1991. Founder effect of a prevalent phenyl-ketoneuria mutation in the Oriental population. *Proc. Natl. Acad. Sci. USA* 88: 2146-2150.
5. Kappock, T.J., et al. 1995. Spectroscopic and kinetic properties of unphosphorylated rat hepatic phenylalanine hydroxylase expressed in *Escherichia coli*. Comparison of resting and activated states. *J. Biol. Chem.* 270: 30532-30544.
6. Fusetti, F., et al. 1998. Structure of tetrameric human phenylalanine hydroxylase and its implications for phenylketonuria. *J. Biol. Chem.* 273: 16962-16967.

CHROMOSOMAL LOCATION

Genetic locus: PAH (human) mapping to 12q23.2.

PRODUCT

PAH 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PAH shRNA Plasmid (h): sc-41528-SH and PAH shRNA (h) Lentiviral Particles: sc-41528-V as alternate gene silencing products.

For independent verification of PAH (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-41528A, sc-41528B and sc-41528C.

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

PAH siRNA (h) is recommended for the inhibition of PAH 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 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

PAH (H-2): sc-271258 is recommended as a control antibody for monitoring of PAH 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 PAH gene expression knockdown using RT-PCR Primer: PAH (h)-PR: sc-41528-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.