SANTA CRUZ BIOTECHNOLOGY, INC.

PBGD siRNA (h): sc-45702

BACKGROUND

PBGD (porphobilinogen deaminase), also designated Hydroxymethylbilane synthase, is a cytoplasmic enzyme found in the heme synthesis pathway. PBGD belongs to the HMBS (hydroxymethylbilane synthase) family. Deficiency of PBGD causes errors in pyrrole metabolism which in turn leads to an inherited autosomal disorder called acute intermittent porphyria (AIP) which is characterized by acute attacks of neurological dysfunctions with hypertension, tachycardia, peripheral neurologic disturbances, abdominal pain and excessive amounts of aminolevulinic acid and porphobilinogen in the urine.

REFERENCES

1. Grandchamp, B., et al. 1987. Tissue-specific expression of porphobilinogen deaminase. Two isoenzymes from a single gene. Eur. J. Biochem. 162: 105-110.
2. Mustajoki, S., et al. 2000. Acute intermittent porphyria: expression of mutant and wild-type porphobilinogen deaminase in COS-1 cells. Mol. Med. 6: 670-679.
3. Schneider-Yin, X., et al. 2004. Mutation hotspots in the human porphobilinogen deaminase gene: recurrent mutations G111R and R173Q occurring at CpG motifs. J. Inherit. Metab. Dis. 27: 625-631.
4. Neuvians, T.P., et al. 2005. Standardization strategy for quantitative PCR in human seminoma and normal testis. J. Biotechnol. 117: 163-171.
5. von und zu Fraunberg, M., et al. 2005. Clinical and biochemical characteristics and genotype-phenotype correlation in 143 Finnish and Russian patients with acute intermittent porphyria. Medicine 84: 35-47.
6. Sheppard, L. and Dorman, T. 2005. Anesthesia in a child with homozygous porphobilinogen deaminase deficiency: a severe form of acute intermittent porphyria. Paediatr. Anaesth. 15: 426-428.
7. SWISS-PROT/TrEMBL (P08397). World Wide Web URL: http://www.expasy.ch/sprot/sprot-top.html

CHROMOSOMAL LOCATION

Genetic locus: HMBS (human) mapping to 11q23.3.

PRODUCT

PBGD 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 PBGD shRNA Plasmid (h): sc-45702-SH and PBGD shRNA (h) Lentiviral Particles: sc-45702-V as alternate gene silencing products.

For independent verification of PBGD (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-45702A, sc-45702B and sc-45702C.

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 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

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

PBGD (E-9): sc-166743 is recommended as a control antibody for monitoring of PBGD 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 PBGD gene expression knockdown using RT-PCR Primer: PBGD (h)-PR: sc-45702-PR (20 µl). 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.