SANTA CRUZ BIOTECHNOLOGY, INC.

PBGS siRNA (h): sc-61385

BACKGROUND

PGBS (porphobilinogen synthase), an enzyme that belongs to the ALADH family, is composed of eight identical subunits and catalyzes the condensation of two molecules of δ-aminolevulinate to form porphobilinogen, a precursor of heme, cytochromes and other hemoproteins. It also catalyzes the second step in the porphyrin and heme biosynthetic pathway in which zinc is essential for enzymatic activity. PGBS is inhibited by lead. A defect in the gene encoding PGBS, ALAD, can cause increased sensitivity to lead poisoning and acute hepatic porphyria, a group of inherited disorders caused by partial enzyme defects in heme biosynthesis, which includes acute intermittent porphyria, variegate porphyria and hereditary coproporphyria. There are two common alleles of ALAD, ALAD*2 and ALAD*1. When exposed to environmental lead, individuals heterozygous or homozygous for ALAD*2 Asn 59 have significantly higher blood lead levels than do ALAD*1 Lys 59 homozygotes.

REFERENCES

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2. Gabriel, D., et al. 2005. Human erythrocyte δ-aminolevulinate dehydratase inhibition by monosaccharides is not mediated by oxidation of enzyme sulfhydryl groups. Cell Biol. Int. 29: 669-674.
3. Hernandez, A.F., et al. 2005. Changes in erythrocyte enzymes in humans long-term exposed to pesticides: influence of several markers of individual susceptibility. Toxicol. Lett. 159: 13-21.
4. Farina, M., et al. 2005. Hematological changes in rats chronically exposed to oral aluminum. Toxicology 209: 29-37.
5. Aisemberg, J., et al. 2005. Comparative study on two freshwater invertebrates for monitoring environmental lead exposure. Toxicology 210: 45-53.
6. Lee, M.K., et al. 2005. Du-zhong *(Eucommia ulmoides Oliv.)* cortex water extract alters heme biosynthesis and erythrocyte antioxidant defense system in lead-administered rats. J. Med. Food 8: 86-92.
7. Roza, T., et al. 2005. 2,3-Dimercapto-1-propanol does not alter the porphobilinogen synthase inhibition but decreases the mercury content in liver and kidney of suckling rats exposed to HgCl2. Basic Clin. Pharmacol. Toxicol. 96: 302-308.

CHROMOSOMAL LOCATION

Genetic locus: ALAD (human) mapping to 9q32.

PRODUCT

PBGS siRNA (h) is a target-specific 19-25 nt siRNA 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 PBGS shRNA Plasmid (h): sc-61385-SH and PBGS shRNA (h) Lentiviral Particles: sc-61385-V as alternate gene silencing products.

RESEARCH USE

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

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

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

PBGS (A-7): sc-271585 is recommended as a control antibody for monitoring of PBGS 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 PBGS gene expression knockdown using RT-PCR Primer: PBGS (h)-PR: sc-61385-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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