

H6PD siRNA (h): sc-62431

BACKGROUND

H6PD (hexose-6-phosphate dehydrogenase, GDH/6PGL endoplasmic bifunctional protein) is a 789 amino acid protein encoded by the human gene H6PD. The N-terminal section of H6PD belongs to the glucose-6-phosphate dehydrogenase family, while the C-terminal section belongs to the glucosamine/galactosamine-6-phosphate isomerase family, 6-phosphogluconolactonase subfamily. H6PD is responsible primarily for the oxidation of glucose-6-phosphate and glucose. It also oxidizes other hexose-6-phosphates. H6PD catalyzes the conversion of glucose 6-phosphate to 6-phosphogluconolactone within the lumen of the endoplasmic reticulum, thereby generating reduced nicotinamide adenine dinucleotide phosphate. Reduced nicotinamide adenine dinucleotide phosphate is a necessary cofactor for the reductase activity of 11 β -hydroxysteroid dehydrogenase type 1, which converts hormonally inactive cortisone to active cortisol (in rodents, 11-dehydrocorticosterone to corticosterone).

REFERENCES

1. Draper, N., et al. 2003. Mutations in the genes encoding 11 β -hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase interact to cause cortisone reductase deficiency. *Nat. Genet.* 34: 434-439.
2. San Millán, J.L., et al. 2005. A study of the hexose-6-phosphate dehydrogenase gene R453Q and 11 β -hydroxysteroid dehydrogenase type 1 gene 83557insA polymorphisms in the polycystic ovary syndrome. *J. Clin. Endocrinol. Metab.* 90: 4157-4162.
3. White, P.C. 2005. Genotypes at 11 β -hydroxysteroid dehydrogenase type 11B1 and hexose-6-phosphate dehydrogenase loci are not risk factors for apparent cortisone reductase deficiency in a large population-based sample. *J. Clin. Endocrinol. Metab.* 90: 5880-5883.
4. Lavery, G.G., et al. 2006. Hexose-6-phosphate dehydrogenase knock-out mice lack 11 β -hydroxysteroid dehydrogenase type 1-mediated glucocorticoid generation. *J. Biol. Chem.* 281: 6546-6551.
5. Crooke, A., et al. 2006. Transient silencing of *Plasmodium falciparum* bifunctional glucose-6-phosphate dehydrogenase-6-phosphogluconolactonase. *FEBS J.* 273: 1537-1546.
6. Odermatt, A., et al. 2006. Why is 11 β -hydroxysteroid dehydrogenase type 1 facing the endoplasmic reticulum lumen? Physiological relevance of the membrane topology of 11 β -HSD1. *Mol. Cell. Endocrinol.* 248: 15-23.

CHROMOSOMAL LOCATION

Genetic locus: H6PD (human) mapping to 1p36.22.

PRODUCT

H6PD 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 H6PD shRNA Plasmid (h): sc-62431-SH and H6PD shRNA (h) Lentiviral Particles: sc-62431-V as alternate gene silencing products.

For independent verification of H6PD (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-62431A, sc-62431B and sc-62431C.

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

H6PD siRNA (h) is recommended for the inhibition of H6PD 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

H6PD (C-10): sc-377180 is recommended as a control antibody for monitoring of H6PD 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 H6PD gene expression knockdown using RT-PCR Primer: H6PD (h)-PR: sc-62431-PR (20 μ l, 548 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.