SANTA CRUZ BIOTECHNOLOGY, INC.

GPI siRNA (r): sc-270226

BACKGROUND

Glucose-6-phosphate isomerase (GPI) has many other names, including phosphohexose isomerase (PHI), neuroleukin (NLK) and spermantigen-36 (SA-36). GPI is a cytoplasmic homodimer belonging to the GPI family. It is a neurotrophic factor for spinal and sensory neurons and is involved in glycolysis and gluconeogenesis. Defects or mutations in GPI can cause hereditary nonspherocytic hemolytic anemia (HA), hydrops fetalis, immediate neonatal death and neurological impairment.

REFERENCES

1. Beutler, E., et al. 1997. Glucosephosphate isomerase (GPI) deficiency mutations associated with hereditary nonspherocytic hemolytic anemia (HNSHA). Blood Cells Mol. Dis. 23: 402-409.
2. Kugler, W., et al. 1998. Molecular basis of neurological dysfunction coupled with haemolytic anaemia in human glucose-6-phosphate isomerase (GPI) deficiency. Hum. Genet. 103: 450-454.
3. Schulz, L.C., et al. 2003. Glucose-6-phosphate isomerase is necessary for embryo implantation in the domestic ferret. Proc. Natl. Acad. Sci. USA 100: 8561-8566.
4. Muraki, Y., et al. 2004. Glucose-6-phosphate isomerase variants play a key role in the generation of anti-GPI antibodies: possible mechanism of autoantibody production. Biochem. Biophys. Res. Commun. 323: 518-522.
5. Graham Solomons, J.T., et al. 2004. The crystal structure of mouse phosphoglucose isomerase at 1.6A resolution and its complex with glucose 6-phosphate reveals the catalytic mechanism of sugar ring opening. J. Mol. Biol. 342: 847-860.
6. Schubert, D., et al. 2004. Immunization with glucose-6-phosphate isomerase induces T cell-dependent peripheral polyarthritis in genetically unaltered mice. J. Immunol. 172: 4503-4509.
7. SWISS-PROT/TrEMBL (P06744). World Wide Web URL: http://www.expasy.ch/sprot/sprot-top.html
8. http://harvester.embl.de/harvester/P067/P06744.htm

CHROMOSOMAL LOCATION

Genetic locus: Gpi (rat) mapping to 1q21.

PRODUCT

GPI siRNA (r) 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 GPI shRNA Plasmid (r): sc-270226-SH and GPI shRNA (r) Lentiviral Particles: sc-270226-V as alternate gene silencing products.

For independent verification of GPI (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270226A, sc-270226B and sc-270226C.

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

GPI siRNA (r) is recommended for the inhibition of GPI expression in rat 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

GPI (H-10): sc-365066 is recommended as a control antibody for monitoring of GPI 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 GPI gene expression knockdown using RT-PCR Primer: GPI (r)-PR: sc-270226-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.

PROTOCOLS

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