

PGAM1 siRNA (m): sc-62782

BACKGROUND

Members of the PGAM (phosphoglycerate mutase) family of proteins are important components of glucose and 2,3-BPGA (2,3-bisphosphoglycerate) metabolism. They are responsible for catalyzing the transfer of phospho groups between the carbon atoms of phosphoglycerates. In mammals there are two types of PGAM isozymes: PGAM1 (also known as PGAMB) and PGAM2 (also known as PGAMA). In the cell, PGAM1 and PGAM2 exist as either homodimers or heterodimers and are responsible for the interconversion of 3-phosphoglycerate and 2-phosphoglycerate. PGAM2 homodimers are expressed in skeletal muscle, mature sperm cells and heart; PGAM1 homodimers are found in most other tissues; and PGAM1/PGAM2 heterodimers are found exclusively in the heart. PGAM4, also known as PGAM3, is a protein formerly considered to be specific to humans. Initially the PGAM4 gene was described as a pseudogene but it is now known to encode a functional protein at least 25 million years old. The gene encoding PGAM4 is believed to have originated by retrotransposition, with the original copy being the PGAM1 gene.

REFERENCES

1. Zhang, J., et al. 2001. Mouse phosphoglycerate mutase M and B isozymes: cDNA cloning, enzyme activity assay and mapping. *Gene* 264: 273-279.
2. Betrán, E., et al. 2002. Evolution of the phosphoglycerate mutase processed gene in human and chimpanzee revealing the origin of a new primate gene. *Mol. Biol. Evol.* 19: 654-663.
3. Shalom-Barak, T., et al. 2002. A p21-activated kinase-controlled metabolic switch up-regulates phagocyte NADPH oxidase. *J. Biol. Chem.* 277: 40659-40665.
4. Saavedra, E., et al. 2005. Glycolysis in *Entamoeba histolytica*. Biochemical characterization of recombinant glycolytic enzymes and flux control analysis. *FEBS J.* 272: 1767-1783.
5. Evans, M.J., et al. 2005. Target discovery in small-molecule cell-based screens by *in situ* proteome reactivity profiling. *Nat. Biotechnol.* 23: 1303-1307.
6. de Atauri, P., et al. 2005. Characterization of the first described mutation of human red blood cell phosphoglycerate mutase. *Biochim. Biophys. Acta* 1740: 403-410.

CHROMOSOMAL LOCATION

Genetic locus: Pgam1 (mouse) mapping to 19 C3.

PRODUCT

PGAM1 siRNA (m) 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 PGAM1 shRNA Plasmid (m): sc-62782-SH and PGAM1 shRNA (m) Lentiviral Particles: sc-62782-V as alternate gene silencing products.

For independent verification of PGAM1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-62782A, sc-62782B and sc-62782C.

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

PGAM1 siRNA (m) is recommended for the inhibition of PGAM1 expression in mouse 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

PGAM1/4 (D-5): sc-365677 is recommended as a control antibody for monitoring of PGAM1 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 PGAM1 gene expression knockdown using RT-PCR Primer: PGAM1 (m)-PR: sc-62782-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.