

PGK1 siRNA (h): sc-36215

BACKGROUND

Phosphoglycerate kinases 1/2 (PGK1/2, ATP:3-phospho-D-glycerate 1-phosphotransferase, EC 2.7.2.3) are somatically expressed, glycolytic enzymes that catalyze the transfer of a phosphoryl group from the acyl phosphate of 1,3-bisphosphoglycerate to ADP, thereby forming ATP and 3-phosphoglycerate. The human PGK gene is interrupted by 10 introns and spans 23 kilobases, and is X chromosome-linked at position Xq21.1, a region implicated in prostate cancer, androgen insensitivity, perineal hypospadias, and other genetic abnormalities. In addition to influencing glycolysis, the PGK1 is secreted by tumor cells and contributes to proliferative angiogenic processes as a disulfide reductase. PGK1 mediated reduction of disulphide bonds in the serine proteinase plasmin initiates the release of the tumor blood vessel inhibitor angiostatin, an event that is critical for blood vessel formation or angiogenesis in tumor expansion and metastasis.

REFERENCES

1. Michelson, A.M., et al. 1985. Structure of the human phosphoglycerate kinase gene and the intron-mediated evolution and dispersal of the nucleotide-binding domain. *Proc. Natl. Acad. Sci. USA* 82: 6965-6969.
2. Ogino, T., et al. 1999. Involvement of a cellular glycolytic enzyme, phosphoglycerate kinase, in Sendai virus transcription. *J. Biol. Chem.* 274: 35999-36008.
3. Riley, D.E., et al. 1999. A hemizygous short tandem repeat polymorphism 3' to the human phosphoglycerate kinase gene. *Mol. Biol. Rep.* 26: 159-165.
4. Lay, A.J., et al. 2000. Phosphoglycerate kinase acts in tumour angiogenesis as a disulphide reductase. *Nature* 408: 869-873.

CHROMOSOMAL LOCATION

Genetic locus: PGK1 (human) mapping to Xq21.1.

PRODUCT

PGK1 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 PGK1 shRNA Plasmid (h): sc-36215-SH and PGK1 shRNA (h) Lentiviral Particles: sc-36215-V as alternate gene silencing products.

For independent verification of PGK1 (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-36215A, sc-36215B and sc-36215C.

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

PGK1 siRNA (h) is recommended for the inhibition of PGK1 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

PGK1 (14): sc-130335 is recommended as a control antibody for monitoring of PGK1 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 PGK1 gene expression knockdown using RT-PCR Primer: PGK1 (h)-PR: sc-36215-PR (20 μ l, 473 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ji, E.H., et al. 2017. Metabolomic analysis of human oral cancer cells with adenylate kinase 2 or phosphorylate glycerol kinase 1 inhibition. *J. Cancer* 8: 298-304.

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.