SANTA CRUZ BIOTECHNOLOGY, INC.

GAPDH-2 siRNA (m): sc-40627

BACKGROUND

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), also called uracil DNA glycosylase, catalyzes the reversible oxidative phosphorylation of glyceraldehyde-3-phosphate in the presence of inorganic phosphate and nicotinamide adenine dinucleotide (NAD), an important energy-yielding step in carbohydrate metabolism. While GAPDH has long been recognized as playing an integral role in glycolysis, additional functions of GAPDH include acting as a uricil DNA glycosylase, activating transcription, binding RNA and involvement in nuclear RNA export, DNA replication and DNA repair. Expression of GAPDH is upregulated in liver, lung and prostate cancers. GAPDH translocates to the nucleus during apoptosis. GAPDH complexes with neuronal proteins implicated in human neuro-degenerative disorders including the β-Amyloid precursor, Huntingtin and other triplet repeat neuronal disorder proteins.

REFERENCES

1. Meyer-Siegler, K., et al. 1991. A human nuclear uracil DNA glycosylase is the 37-kDa subunit of glyceraldehyde-3-phosphate dehydrogenase. Proc. Natl. Acad. Sci. USA 88: 8460-8464.
2. Rondinelli, R.H., et al. 1997. Increased glyceraldehyde-3-phosphate dehydrogenase gene expression in late pathological stage human prostate cancer. Prostate Cancer Prostatic Dis. 1: 66-72.
3. Eyschen, J., et al. 1999. Engineered glycolytic glyceraldehyde-3-phosphate dehydrogenase binds the anti conformation of NAD^+ nicotinamide but does not experience A-specific hydride transfer. Arch. Biochem. Biophys. 364: 219-227.
4. Sirover, M.A. 1999. New insights into an old protein: the functional diversity of mammalian glyceraldehyde-3-phosphate dehydrogenase. Biochim. Biophys. Acta 1432: 159-184.
5. Berry, M.D. and Boulton, A.A. 2000. Glyceraldehyde-3-phosphate dehydrogenase and apoptosis. J. Neurosci. Res. 60: 150-154.
6. Tatton, W.G., et al. 2000. Glyceraldehyde-3-phosphate dehydrogenase in neurodegeneration and apoptosis signaling. J. Neural Transm. Suppl. 60: 77-100.

CHROMOSOMAL LOCATION

Genetic locus: Gapdhs (mouse) mapping to 7 B1.

PRODUCT

GAPDH-2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GAPDH-2 shRNA Plasmid (m): sc-40627-SH and GAPDH-2 shRNA (m) Lentiviral Particles: sc-40627-V as alternate gene silencing products.

For independent verification of GAPDH-2 (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-40627A, sc-40627B and sc-40627C.

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

GAPDH-2 siRNA (m) is recommended for the inhibition of GAPDH-2 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 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

GAPDH-2 (2E3-2E10): sc-293335 is recommended as a control antibody for monitoring of GAPDH-2 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 GAPDH-2 gene expression knockdown using RT-PCR Primer: GAPDH-2 (m)-PR: sc-40627-PR (20 µl, 494 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.