SANTA CRUZ BIOTECHNOLOGY, INC.

KGA siRNA (h): sc-105592

BACKGROUND

Glutamine is an important molecule involved in several cellular functions, including nitrogen and carbon transport, hepatic urea synthesis, renal ammoniagenesis, and gluconeogenesis. Glutamine is catabolized by either the liver-type (LGA) or kidney-type (KGA) glutaminase. KGA is mitochondrial specific protein whose expression in kidney is increased during metabolic acidosis. This process is mediated by an 8-base AU-sequence in KGA that functions as a pH-response element. The human KGA gene maps to chromosome 2, and produces three isoforms, designated KGA, GAC, and GAM, by alternative splicing. KGA is synthesized as a cytosolic protein that is transported to the mitochondria as an intermediate protein, and is further cleaved into the KGA isoform and the GAC isoform. The processing of the GAM isoform is unclear. The KGA isoform is abundant in brain and kidney, while the GAC isoform is principally expressed in cardiac muscle and pancreas. The GAM isoform is solely expressed in cardiac and skeletal muscle.

REFERENCES

1. Curthoys, N.P. and Watford, M. 1995. Regulation of glutaminase activity and glutamine metabolism. Annu. Rev. Nutr. 15: 133-159.
2. Srinivasan, M., et al. 1995. *In vitro* characterization of the mitochondrial processing and the potential function of the 68-kDa subunit of renal glutaminase. J. Biol. Chem. 270: 1185-1190.
3. Srinivasan, M., et al. 1995. Role of the N-terminal 118 amino acids in the processing of the rat renal mitochondrial glutaminase precursor. J. Biol. Chem. 270: 1191-1197.

CHROMOSOMAL LOCATION

Genetic locus: GLS (human) mapping to 2q32.2.

PRODUCT

KGA 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see KGA shRNA Plasmid (h): sc-105592-SH and KGA shRNA (h) Lentiviral Particles: sc-105592-V as alternate gene silencing products.

For independent verification of KGA (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-105592A, sc-105592B and sc-105592C.

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

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

KGA (IJ-2): sc-100533 is recommended as a control antibody for monitoring of KGA 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 KGA gene expression knockdown using RT-PCR Primer: KGA (h)-PR: sc-105592-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lee, J.S., et al. 2016. Dual targeting of glutaminase 1 and thymidylate synthase elicits death synergistically in NSCLC. Cell Death Dis. 7: e2511.
2. Choudhury, M., et al. 2020. SIRT7-mediated modulation of glutaminase 1 regulates TGF-β-induced pulmonary fibrosis. FASEB J. 34: 8920-8940.
3. Bando, H., et al. 2021. Depletion of intracellular glutamine pools triggers *Toxoplasma gondii* stage conversion in human glutamatergic neurons. Front. Cell. Infect. Microbiol. 11: 788303.

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.