SANTA CRUZ BIOTECHNOLOGY, INC.

LGA siRNA (h): sc-96242

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The Power to Question

BACKGROUND

LGA (L-glutamine amidohydrolase), also known as GLS2 (glutaminase 2 (liver, mitochondrial)), GLS or GA, is a 602 amino acid protein that localizes to the mitochondrion and contains two ANK repeats. Expressed in brain, liver and pancreas, LGA functions as a mitochondrial phosphate-activated glutaminase that catalyzes the hydrolysis of glutamine to glutamate and ammonia. LGA is overexpressed in breast cancer cell lines, suggesting a role for LGA in tumorigenesis. The gene encoding LGA maps to human chromosome 12, which encodes over 1,100 genes and comprises approximately 4.5% of the human genome. Chromosome 12 is associated with a variety of diseases and afflictions, including hypochondrogenesis, achondrogenesis, Kniest dysplasia, Noonan syndrome and Trisomy 12p, which causes facial developmental defects and seizure disorders.

REFERENCES

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3. Olalla, L., et al. 2001. The C-terminus of human glutaminase L mediates association with PDZ domain-containing proteins. FEBS Lett. 488: 116-122.
4. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 606365. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Olalla, L., et al. 2002. Nuclear localization of L-type glutaminase in mammalian brain. J. Biol. Chem. 277: 38939-38944.
6. Perez-Gómez, C., et al. 2003. Genomic organization and transcriptional analysis of the human l-glutaminase gene. Biochem. J. 370: 771-784.
7. Zhao, J., et al. 2004. Mitochondrial glutaminase enhances extracellular glutamate production in HIV-1-infected macrophages: linkage to HIV-1 associated dementia. J. Neurochem. 88: 169-180.

CHROMOSOMAL LOCATION

Genetic locus: GLS2 (human) mapping to 12q13.3.

PRODUCT

LGA 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 LGA shRNA Plasmid (h): sc-96242-SH and LGA shRNA (h) Lentiviral Particles: sc-96242-V as alternate gene silencing products.

For independent verification of LGA (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-96242A, sc-96242B and sc-96242C.

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

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

LGA (175I2M): sc-517626 is recommended as a control antibody for monitoring of LGA 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 LGA gene expression knockdown using RT-PCR Primer: LGA (h)-PR: sc-96242-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.