



GIF siRNA (h): sc-97044

BACKGROUND

Vitamin B12, also known as cobalamin, is a water-soluble vitamin that is required for formation of red blood cells and for normal functioning of the nervous system. Used to regenerate folate concentrations within the body, vitamin B12 is part of a biochemical pathway that synthesizes the DNA component thymine. GIF (gastric intrinsic factor) is a 417 amino acid secreted glycoprotein that is expressed in gastric mucosa. GIF is produced by parietal cells of the stomach and is necessary for absorption of vitamin B12. Once vitamin B12 is bound by GIF, it can be absorbed in the terminal ileum via the receptor cubilin. Pernicious anemia, an autoimmune disease that destroys parietal cells within the stomach, results from lack of intrinsic factor leading to malabsorption of vitamin B12 and megaloblastic anemia, which is characterized by large immature and dysfunctional red blood cells. Defects in the GIF gene itself is the cause of hereditary intrinsic factor deficiency, which is also characterized by subsequent megaloblastic anemia.

REFERENCES

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- Seetharam, B., et al. 1999. Cellular import of cobalamin (Vitamin B-12). *J. Nutr.* 129: 1761-1764.
- Seetharam, B. and Yammani, R.R. 2003. Cobalamin transport proteins and their cell-surface receptors. *Expert Rev. Mol. Med.* 5: 1-18.
- Gordon, M.M., et al. 2004. A genetic polymorphism in the coding region of the gastric intrinsic factor gene (GIF) is associated with congenital intrinsic factor deficiency. *Hum. Mutat.* 23: 85-91.
- Tanner, S.M., et al. 2005. Hereditary juvenile cobalamin deficiency caused by mutations in the intrinsic factor gene. *Proc. Natl. Acad. Sci. USA* 102: 4130-4133.
- Rufenacht, P., et al. 2008. Vitamin B12 deficiency: a challenging diagnosis and treatment. *Rev. Med. Suisse.* 4: 2212-2216.

CHROMOSOMAL LOCATION

Genetic locus: GIF (human) mapping to 11q12.1.

PRODUCT

GIF 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 GIF shRNA Plasmid (h): sc-97044-SH and GIF shRNA (h) Lentiviral Particles: sc-97044-V as alternate gene silencing products.

For independent verification of GIF (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-97044A, sc-97044B and sc-97044C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

GIF (D-6): sc-514523 is recommended as a control antibody for monitoring of GIF 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 GIF gene expression knockdown using RT-PCR Primer: GIF (h)-PR: sc-97044-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.