

α -gal A siRNA (h): sc-105019

BACKGROUND

α -galactosidase A (α -gal A) functions as a lysosomal hydrolase. α -gal A forms an active homodimer that acts upon a glycolipid substrate, globotriaosylceramide ($G_{\beta 3}$). Inherited mutations in this gene cause an X-linked recessive glycolipid storage disorder known as Fabry's disease. In Fabry patients, α -gal A deficiencies lead to an accumulation of $G_{\beta 3}$ in the body. The numerous clinical manifestations of the disease include renal and cardiac impairment, severe pain in the extremities and cutaneous lesions known as angiokeratomas. Enzyme replacement therapy using recombinant α -gal A effectively treats the symptoms of Fabry disease.

REFERENCES

1. Kint, J.A. 1970. Fabry's disease: α -galactosidase deficiency. *Science* 167: 1268-1269.
2. Sweatman, A.K., et al. 1994. Physical mapping in the region of the Bruton's tyrosine kinase and α -galactosidase A gene loci in proximal Xq22. *Hum. Genet.* 94: 624-628.
3. Schiffmann, R., et al. 2000. Infusion of α -galactosidase A reduces tissue globotriaosylceramide storage in patients with Fabry disease. *Proc. Natl. Acad. Sci. USA* 97: 365-370.
4. Ioannou, Y.A., et al. 2001. Fabry disease: preclinical studies demonstrate the effectiveness of α -galactosidase A replacement in enzyme-deficient mice. *Am. J. Hum. Genet.* 68: 14-25.
5. Eng, C.M., et al. 2001. A phase 1/2 clinical trial of enzyme replacement in fabry disease: pharmacokinetic, substrate clearance, and safety studies. *Am. J. Hum. Genet.* 68: 711-722.

CHROMOSOMAL LOCATION

Genetic locus: GLA (human) mapping to Xq22.1.

PRODUCT

α -gal A 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 α -gal A shRNA Plasmid (h): sc-105019-SH and α -gal A shRNA (h) Lentiviral Particles: sc-105019-V as alternate gene silencing products.

For independent verification of α -gal A (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-105019A, sc-105019B and sc-105019C.

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

α -gal A siRNA (h) is recommended for the inhibition of α -gal A 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

α -gal A (C6): sc-517442 is recommended as a control antibody for monitoring of α -gal A 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 α -gal A gene expression knockdown using RT-PCR Primer: α -gal A (h)-PR: sc-105019-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Squillaro, T., et al. 2017. Impact of lysosomal storage disorders on biology of mesenchymal stem cells: evidences from *in vitro* silencing of glucocerebrosidase (GBA) and α -galactosidase A (GLA) enzymes. *J. Cell. Physiol.* 232: 3454-3467.

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.