SANTA CRUZ BIOTECHNOLOGY, INC.

β-glucuronidase siRNA (h): sc-44458

BACKGROUND

The enzyme β-glucuronidase catalyzes the conversion of β-D-glucuronoside and water to an alcohol and D-glucuronate. Deficiency of β-glucuronidase is the cause of the human lysosomal storage disorder mucopolysaccharidosis type VII (MPS VII). Specifically, two residues appear important for catalytic activity: Glu 451 and Glu 540. Mutations at these sites affect the overall structure of the protein, which normally consists of a homotetramer with each promoter including a jelly roll barrel, an immunoglobulin constant domain and a TIM barrel. Regulation of β-glucuronidase activity may play a role in tumorigenesis and the invasiveness of a number of cancers, and is also an important factor in the development of functional prodrugs that require the cleavage of an active cytostatic by endogenous enzymes for antitumor activity.

REFERENCES

1. Himeno Mnishimura, Y., et al. 1976. Purification and characterization of microsomal and lysosomal β-glucuronidase from rat liver by use of immunoaffinity chromatography. Eur. J. Biochem. 70: 349-359.
2. Gupta, G.S. and Singh, G.P. 1983. Isolation and characterization of the major form of β-glucuronidase from human seminal plasma. Biochim. Biophys. Acta 748: 398-404.
3. Varma, R., et al. 1983. β-glucuronidase in sera of patients with epileptic seizure activity, diabetes and some other disease states. Neurosci. Lett. 39: 105-111.
4. Guise, K.S., et al. 1985. Isolation and expression in *Escherichia coli* of a cDNA clone encoding human β-glucuronidase. Gene 34: 105-110.
5. Watson, G., et al. 1985. Properties of rat and mouse β-glucuronidase mRNA and cDNA, including evidence for sequence polymorphism and genetic regulation of mRNA levels. Gene 36: 15-25.
6. Jain, S., et al. 1996. Structure of human β-glucuronidase reveals candidate lysosomal targeting and active-site motifs. Nat. Struct. Biol. 3: 375-381.
7. Vervoort, R., et al. 1998. Low β-glucuronidase enzyme activity and mutations in the human β-glucuronidase gene in mild mucopolysaccharidosis type VII, pseudodeficiency and a heterozygote. Hum. Genet. 102: 69-78.

CHROMOSOMAL LOCATION

Genetic locus: GUSB (human) mapping to 7q11.21.

PRODUCT

β-glucuronidase 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 β-glucuronidase shRNA Plasmid (h): sc-44458-SH and β-glucuronidase shRNA (h) Lentiviral Particles: sc-44458-V as alternate gene silencing products.

For independent verification of β-glucuronidase (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-44458A, sc-44458B and sc-44458C.

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

β-glucuronidase siRNA (h) is recommended for the inhibition of β-glucuronidase 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

β-glucuronidase (E-11): sc-374629 is recommended as a control antibody for monitoring of β-glucuronidase 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 β-glucuronidase gene expression knockdown using RT-PCR Primer: β-glucuronidase (h)-PR: sc-44458-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.