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β -Gal siRNA (h): sc-43792



The Power to Question

BACKGROUND

The human β -galactosidase gene, known as the LacZ gene, maps to chromosome 3p21.33 and encodes a 677 amino acid protein with an optimum functional pH range of 6 to 8. Catalytically active β -galactosidase (β -Gal) is a tetramer of four identical subunits, each with an active site, which can independently catalyze the cleavage of terminal galactose. Monovalent cations have a stimulatory effect on the enzymatic reaction, which likely involves a galactosyl-enzyme complex intermediate. β -Gals are widespread in animals, microorganisms and plants. The LacZ gene is widely used as a reporter gene with a variety of colored or fluorescent compounds capable of being produced from appropriate substrates, such as Xgal, which produces a blue color. For this reason, LacZ is incorporated into numerous plasmid vectors as a marker.

REFERENCES

- Oshima, A., et al. 1988. Cloning, sequencing, and expression of cDNA for human β -galactosidase. *Biochem. Biophys. Res. Commun.* 157: 238-244.
- Morreau, H., et al. 1989. Alternative splicing of β -galactosidase mRNA generates the classic lysosomal enzyme and a β -galactosidase-related protein. *J. Biol. Chem.* 264: 20655-20663.
- Draber, P., et al. 1992. Monoclonal antibodies to *Escherichia coli* β -galactosidase and their use for detection and purification of recombinant expression products. *Hybridoma* 11: 385-390.
- Slavickova, A., et al. 1993. A novel panel of monoclonal antibodies against β -galactosidase of *Escherichia coli* and its versatility for detection of recombinant expression products. *Folia Biol.* 38: 350-357.

CHROMOSOMAL LOCATION

Genetic locus: GLB1 (human) mapping to 3p22.3.

PRODUCT

β -Gal 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 β -Gal shRNA Plasmid (h): sc-43792-SH and β -Gal shRNA (h) Lentiviral Particles: sc-43792-V as alternate gene silencing products.

For independent verification of β -Gal (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-43792A, sc-43792B and sc-43792C.

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

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

β -Gal (B-12): sc-377257 is recommended as a control antibody for monitoring of β -Gal 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 β -Gal gene expression knockdown using RT-PCR Primer: β -Gal (h)-PR: sc-43792-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

- Szychowski, K.A., et al. 2019. Antiproliferative effect of elastin-derived peptide VGVAPG on SH-SY5Y neuroblastoma cells. *Neurotox. Res.* E-published.

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.