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SANTA CRUZ BIOTECHNOLOGY, INC.

saposin siRNA (h): sc-44456

BACKGROUND

The saposin family includes four structurally related activator proteins, saposin A, B, C and D, that are cleaved from the single precursor protein prosaposin. The gene encoding human prosaposin maps to chromosome 10. Prosaposin is synthesized as a protein that is posttranslationally modified to a shorter form and then further glycosylated to yield a secretory product. This form subsequently undergoes partial proteolysis to produce saposin A, B, C and D. Each saposin family member acts in conjunction with hydrolase enzymes to facilitate the breakdown of glycosphingolipids within the lysosome. The saposins modify the environment of target lipids to make them accessible to the active sites of specific enzymes. Saposin A and C are involved in the hydrolysis of glucosylceramidase and defects in saposin C are linked to Gaucher's disease. Saposin B facilitates the hydrolysis of the sulfate group from cerebroside sulfate and defects in this protein are responsible for a form of metachromatic leukodystropy, a progressive neurodegenerative condition. Saposin D may stimulate the hydrolysis of sphingomyelin and ceramide, but its exact physiological role is not clear.

REFERENCES

1. Schnabel, D., et al. 1991. Mutation in the sphingolipid activator protein 2 in a patient with a variant of Gaucher's disease. FEBS Lett. 284: 57-59.
2. O'Brien, J.S., et al. 1991. Saposin proteins: structure, function, and role in human lysosomal storage disorders. FASEB J. 5: 301-308.
3. Suzuki, Y. 1995. Disorders of sphingolipid activator proteins. Nippon Rinsho 53: 3025-3027.
4. Vaccaro, A.M., et al. 1997. Effect of saposins A and C on the enzymatic hydrolysis of liposomal glucosylceramide. J. Biol. Chem. 272: 16862-16867.
5. Tatti, M., et al. 1999. Structural and membrane-binding properties of saposin D. Eur. J. Biochem. 263: 486-494.

CHROMOSOMAL LOCATION

Genetic locus: PSAP (human) mapping to 10q22.1.

PRODUCT

saposin 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 saposin shRNA Plasmid (h): sc-44456-SH and saposin shRNA (h) Lentiviral Particles: sc-44456-V as alternate gene silencing products.

For independent verification of saposin (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-44456A, sc-44456B and sc-44456C.

PROTOCOLS

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

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

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

saposin C (A-3): sc-374118 is recommended as a control antibody for monitoring of saposin 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 saposin gene expression knockdown using RT-PCR Primer: saposin (h)-PR: sc-44456-PR (20 µl, 599 bp). 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.