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INSIG-2 siRNA (h): sc-45781

BACKGROUND

INSIG-1 and INSIG-2 play distinct roles in a negative-feedback mechanism for cholesterol synthesis. INSIG-1 localizes to the endoplasmic reticulum (ER) and binds the sterol-sensing domain of SREBP cleavage-activating protein (SCAP). Sterol induces INSIG-1 binding to SCAP. INSIG-2, another ER protein, binds SCAP in a sterol-regulated manner. Thus, INSIG-1 and INSIG-2 block the export of SCAP from the ER and ultimately inhibit cholesterol synthesis by preventing the proteolytic processing of SREBPs by Golgi enzymes. The critical role of INSIG-1 and INSIG-2 in cholesterol metabolism may be exploited as a therapeutic effect for hypercholesterolemia.

REFERENCES

1. Peng, Y., et al. 1997. Cloning, human chromosomal assignment and adipose and hepatic expression of the CL-6/INSIG-1 gene. *Genomics* 43: 278-284.
2. Janowski, B.A., et al. 2002. The hypocholesterolemic agent LY295427 upregulates INSIG-1, identifying the INSIG-1 protein as a mediator of cholesterol homeostasis through SREBP. *Proc. Natl. Acad. Sci. USA* 99: 12675-12680.
3. Yabe, D., et al. 2002. INSIG-2, a second endoplasmic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc. Natl. Acad. Sci. USA* 99: 12753-12758.
4. Yabe, D., et al. 2002. Three mutations in sterol-sensing domain of SCAP block interaction with insig and render SREBP cleavage insensitive to sterols. *Proc. Natl. Acad. Sci. USA* 99: 16672-16677.
5. Yang, T., et al. 2002. Crucial step in cholesterol homeostasis: sterols promote binding of SCAP to INSIG-1, a membrane protein that facilitates retention of SREBPs in ER. *Cell* 110: 489-500.

CHROMOSOMAL LOCATION

Genetic locus: INSIG2 (human) mapping to 2q14.2.

PRODUCT

INSIG-2 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 INSIG-2 shRNA Plasmid (h): sc-45781-SH and INSIG-2 shRNA (h) Lentiviral Particles: sc-45781-V as alternate gene silencing products.

For independent verification of INSIG-2 (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-45781A, sc-45781B and sc-45781C.

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.

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

INSIG-2 siRNA (h) is recommended for the inhibition of INSIG-2 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor INSIG-2 gene expression knockdown using RT-PCR Primer: INSIG-2 (h)-PR: sc-45781-PR (20 μ l, 578 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.