

# INSIG-1 siRNA (r): sc-270655

## BACKGROUND

INSIG-1 and INSIG-2 play distinct roles in a negative-feedback mechanism for cholesterol synthesis. INSIG-1 is highly expressed in liver and fibroblast cell lines. 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. INSIG-1 is encoded by the Insulin-induced gene (INSIG-1). INSIG-1 gene expression is suppressed by oxysterols and restored following the introduction of the hypocholesterolemic agent LY295427. The negative feedback mechanism is absent in mutant CHO cells with a point mutation in one SCAP allele within the sterol-sensing domain. The mutant cells constitutively cleave SREBP in the presence of sterols. 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., Schwarz, E.J., Lazar, M.A., Genin, A., Spinner, N.B. and Taub, R. 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. 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., Brown, M.S. and Goldstein, J.L. 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., Xia, Z.P., Adams, C.M. and Rawson, R.B. 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., Espenshade, P.J., Wright, M.E., Yabe, D., Gong, Y., Aebersold, R., Goldstein, J.L. and Brown, M.S. 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: *Insig1* (rat) mapping to 4q11.

## PRODUCT

INSIG-1 siRNA (r) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see INSIG-1 shRNA Plasmid (r): sc-270655-SH and INSIG-1 shRNA (r) Lentiviral Particles: sc-270655-V as alternate gene silencing products.

For independent verification of INSIG-1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270655A, sc-270655B and sc-270655C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

INSIG-1 siRNA (r) is recommended for the inhibition of INSIG-1 expression in rat 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  $\mu$ M in 66  $\mu$ 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

INSIG-1 (A-9): sc-390504 is recommended as a control antibody for monitoring of INSIG-1 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 INSIG-1 gene expression knockdown using RT-PCR Primer: INSIG-1 (r)-PR: sc-270655-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.