

# 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.

## 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  $\mu$ 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.

## 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-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  $\mu$ 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.

## 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  $\mu$ l, 578 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Miyata, S., et al. 2015. Xanthohumol improves diet-induced obesity and fatty liver by suppressing sterol regulatory element-binding protein (SREBP) activation. *J. Biol. Chem.* 290: 20565-72059.
2. Luo, G., et al. 2021. Discovery of an orally active VHL-recruiting PROTAC that achieves robust HMGCR degradation and potent hypolipidemic activity *in vivo*. *Acta Pharm. Sin. B* 11: 1300-1314.
3. Watanabe, Y., et al. 2021. Insulin-induced genes INSIG1 and INSIG2 mediate oxysterol-dependent activation of the PERK/eIF2 $\alpha$ /ATF4 axis. *J. Biol. Chem.* 297: 100989.

## 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.