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# R-Spondin3 siRNA (m): sc-152619

## BACKGROUND

Roof plate-specific Spondins (R-Spondins) are secreted proteins that possess a Furin-like cysteine-rich domain and are involved in regulating  $\beta$ -catenin function. R-Spondin3, also known as RSP03, PWTSR, THSD2 or CRISTIN1, is a 272 amino acid secreted protein that contains one TSP type-1 domain and two furin-like repeats. Expressed ubiquitously with particularly high levels present in placenta, thymus and lymph node, R-Spondin3 functions to activate the  $\beta$ -catenin signaling cascade, ultimately leading to TCF-dependent gene activation. Multiple isoforms of R-Spondin3 exist due to alternative splicing events. The gene encoding R-Spondin3 maps to human chromosome 6, which contains 170 million base pairs and comprises nearly 6% of the human genome. Deletion of a portion of the q arm of chromosome 6 is associated with early onset intestinal cancer, suggesting the presence of a cancer susceptibility locus. Additionally, Porphyria cutanea tarda, Parkinson's disease, Stickler syndrome and a susceptibility to bipolar disorder are all associated with genes that map to chromosome 6.

## REFERENCES

- Adams, J.C. and Tucker, R.P. 2000. The thrombospondin type 1 repeat (TSR) superfamily: diverse proteins with related roles in neuronal development. *Dev. Dyn.* 218: 280-299.
- Chen, J.Z., et al. 2002. Cloning and identification of a cDNA that encodes a novel human protein with thrombospondin type I repeat domain, hPWTSR. *Mol. Biol. Rep.* 29: 287-292.
- Hannah, B.L., et al. 2003. A polymorphism in thrombospondin-1 associated with familial premature coronary heart disease causes a local change in conformation of the  $Ca^{2+}$ -binding repeats. *J. Biol. Chem.* 278: 8929-8934.
- Kim, K.A., et al. 2006. R-Spondin proteins: a novel link to  $\beta$ -catenin activation. *Cell Cycle* 5: 23-26.
- Nam, J.S., et al. 2006. Mouse cristin/R-Spondin family proteins are novel ligands for the frizzled 8 and LRP6 receptors and activate  $\beta$ -catenin-dependent gene expression. *J. Biol. Chem.* 281: 13247-13257.
- Theodorou, V., et al. 2007. MMTV insertional mutagenesis identifies genes, gene families and pathways involved in mammary cancer. *Nat. Genet.* 39: 759-769.

## CHROMOSOMAL LOCATION

Genetic locus: *Rspo3* (mouse) mapping to 10 A4.

## PRODUCT

R-Spondin3 siRNA (m) 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 R-Spondin3 shRNA Plasmid (m): sc-152619-SH and R-Spondin3 shRNA (m) Lentiviral Particles: sc-152619-V as alternate gene silencing products.

For independent verification of R-Spondin3 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-152619A, sc-152619B and sc-152619C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at  $-20^{\circ}$  C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at  $-20^{\circ}$  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

R-Spondin3 siRNA (m) is recommended for the inhibition of R-Spondin3 expression in mouse 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 R-Spondin3 gene expression knockdown using RT-PCR Primer: R-Spondin3 (m)-PR: sc-152619-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60 $^{\circ}$  C and the extension temperature should be 68-72 $^{\circ}$  C.

## SELECT PRODUCT CITATIONS

- Greicius, G., et al. 2018. PDGFR $\alpha^{+}$  pericyptal stromal cells are the critical source of Wnts and RSP03 for murine intestinal stem cells *in vivo*. *Proc. Natl. Acad. Sci. USA* 115: E3173-E3181.

## 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.