

# TRK-T3 siRNA (h): sc-61720

## BACKGROUND

Oncogenic rearrangements of the NTRK1 gene, which encodes the Trk A protein, are frequently detected in thyroid carcinomas. Such rearrangements fuse the NTRK1 tyrosine kinase domain to 5'-end sequences of different genes. TRK-T3 contains 1,412 nucleotides of NTRK1 preceded by 598 nucleotides belonging to TFG (TRK-fused gene), a ubiquitously expressed gene located on chromosome 3. The TRK-T3 protein within the TFG region contains a coiled-coil motif that gives the oncoprotein the capability to form complexes. The cytoplasmic TRK-T3 protein binds to and phosphorylates the Shc and SNT1/FRS2 adaptor proteins, both of which are involved in coupling the receptor tyrosine kinase to the mitogen-activated protein kinase pathway by recruiting Grb2/SOS. SHP-1 also interacts with and downregulates TRK-T3.

## REFERENCES

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3. Ranzi, V., Meakin, S.O., Miranda, C., Mondellini, P., Pierotti, M.A. and Greco, A. 2003. The signaling adapters fibroblast growth are activated by the thyroid TRK oncoproteins. *Endocrinology* 144: 922-928.
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5. Edel, M.J., Shvarts, A., Medema, J.P. and Bernards, R. 2004. An *in vivo* functional genetic screen reveals a role for the TRK-T3 oncogene in tumor progression. *Oncogene* 23: 4959-4965.
6. Roccato, E., Miranda, C., Raho, G., Pagliardini, S., Pierotti, M.A. and Greco, A. 2005. Analysis of SHP-1-mediated down-regulation of the TRK-T3 oncoprotein identifies TRK-fused gene (TFG) as a novel SHP-1-interacting protein. *J. Biol. Chem.* 280: 3382-3389.

## CHROMOSOMAL LOCATION

Genetic locus: TFG (human) mapping to 3q12.2.

## PRODUCT

TRK-T3 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 TRK-T3 shRNA Plasmid (h): sc-61720-SH and TRK-T3 shRNA (h) Lentiviral Particles: sc-61720-V as alternate gene silencing products.

For independent verification of TRK-T3 (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-61720A, sc-61720B and sc-61720C.

## 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

TRK-T3 siRNA (h) is recommended for the inhibition of TRK-T3 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.

## GENE EXPRESSION MONITORING

TRK-T3 (H-11): sc-515054 is recommended as a control antibody for monitoring of TRK-T3 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<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor TRK-T3 gene expression knockdown using RT-PCR Primer: TRK-T3 (h)-PR: sc-61720-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.

## 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.