SANTA CRUZ BIOTECHNOLOGY, INC.

TFG siRNA (m): sc-61721

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 down-regulates TRK-T3.

REFERENCES

1. Greco, A., et al. 1995. The DNA rearrangement that generates the TRK-T3 oncogene involves a novel gene on chromosome 3 whose product has a potential coiled-coil domain. Mol. Cell. Biol. 15: 6118-6127.
2. Roccato, E., et al. 2002. Biological activity of the thyroid TRK-T3 oncogene requires signalling through Shc. Br. J. Cancer 87: 645-53.
3. Ranzi, V., et al. 2003. The signaling adapters fibroblast grow are activated by the thyroid TRK oncoproteins. Endocrinology 144: 922-928.
4. Roccato, E., et al. 2003. Role of TFG sequences outside the coiled-coil domain in TRK-T3 oncogenic activation. Oncogene 22: 807-818.
5. Edel, M.J., et al. 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., et al. 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 (mouse) mapping to 16 C1.1.

PRODUCT

TFG 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TFG shRNA Plasmid (m): sc-61721-SH and TFG shRNA (m) Lentiviral Particles: sc-61721-V as alternate gene silencing products.

For independent verification of TFG (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-61721A, sc-61721B and sc-61721C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

TFG siRNA (m) is recommended for the inhibition of TFG 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 µM in 66 µl. 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 TFG 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 TFG gene expression knockdown using RT-PCR Primer: TFG (m)-PR: sc-61721-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.