

Ret siRNA (r): sc-156121

BACKGROUND

The Ret proto-oncogene is structurally related to the growing family of tyrosine kinase transmembrane receptors and is involved in GDNF signaling. By alternative splicing, two isoforms of the Ret proto-oncogene product are generated. The isoforms differ from each other by having either 9 or 51 carboxy terminal amino acids. The Ret gene products include two glycosylated proteins and, in tunicamycin treated cells, a non-glycosylated protein consistent with the predicted Ret molecular weight based on sequence analysis. Tumor-specific rearrangements of the Ret proto-oncogene have been identified in papillary thyroid carcinomas leading to the formation of different transforming fusion proteins sharing the tyrosine kinase domain of Ret. In contrast to the Ret proto-oncogene, the rearranged forms are constitutively phosphorylated on tyrosine and are translocated from the membrane to the cytoplasm.

REFERENCES

1. Takahashi, M., et al. 1988. Identification of the Ret proto-oncogene products in neuroblastoma and leukemia cells. *Oncogene* 3: 571-578.
2. Tahira, T., et al. 1990. Characterization of Ret proto-oncogene mRNAs encoding two isoforms of the protein product in a human neuroblastoma cell line. *Oncogene* 5: 97-102.
3. Grieco, M., et al. 1990. PTC is a novel rearranged form of the Ret proto-oncogene and is frequently detected *in vivo* in human thyroid papillary carcinomas. *Cell* 60: 557-563.
4. Takahashi, M., et al. 1991. Cloning and expression of the Ret proto-oncogene encoding a tyrosine kinase with two potential transmembrane domains. *Oncogene* 6: 297-301.

CHROMOSOMAL LOCATION

Genetic locus: Ret (rat) mapping to 4q42.

PRODUCT

Ret 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Ret shRNA Plasmid (r): sc-156121-SH and Ret shRNA (r) Lentiviral Particles: sc-156121-V as alternate gene silencing products.

For independent verification of Ret (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-156121A, sc-156121B and sc-156121C.

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

APPLICATIONS

Ret siRNA (r) is recommended for the inhibition of Ret 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 μ M in 66 μ 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

Ret (C-3): sc-365943 is recommended as a control antibody for monitoring of Ret 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 Ret gene expression knockdown using RT-PCR Primer: Ret (r)-PR: sc-156121-PR (20 μ l, 592 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Wan, G. and Too, H.P. 2010. A specific isoform of glial cell line-derived neurotrophic factor family receptor α 1 regulates RhoA expression and glioma cell migration. *J. Neurochem.* 115: 759-770.
2. Spanheimer, P.M., et al. 2014. Distinct pathways regulated by RET and estrogen receptor in luminal breast cancer demonstrate the biological basis for combination therapy. *Ann. Surg.* 259: 793-799.
3. Pertile, R.A.N., et al. 2018. Vitamin D regulation of GDNF/Ret signaling in dopaminergic neurons. *FASEB J.* 32: 819-828.

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.