

CT-R siRNA (r): sc-270180

BACKGROUND

Calcitonin (CT) is a circulating peptide hormone that is secreted from the thyroid and specifically binds to surface calcitonin receptors (CT-R) to regulate calcium homeostasis. These receptors represent a distinct family of seven transmembrane proteins, which include receptors for parathyroid hormone/parathyroid-related peptide, secretin and glucagon. CT-Rs induce intracellular signaling by coupling to multiple heterotrimeric G-proteins, where they then activate several signal transduction pathways involving adenylyl cyclase, phospholipase C and Map kinases. The gene encoding CT-R consists of numerous exons separated by larger introns, which are modified to produce multiple splice variants. These functionally unique isoforms display differential tissue distribution and preferentially associate with specific G-proteins to recruit distinct signaling intermediates. In osteoclasts and embryonic kidney cells, CT binding to the CT-R stimulates the Map kinases Erk1/2 and PKC activity through the phosphorylation of the adaptor proteins Shc and HEF1, and this induction occurs independently from PKA and adenylyl cyclase mediated signaling.

REFERENCES

1. Copp, D.H. 1994. Calcitonin: discovery, development, and clinical application. *Clin. Invest. Med.* 17: 268-277.
2. Kuestner, R.E., et al. 1994. Cloning and characterization of an abundant subtype of the human calcitonin receptor. *Mol. Pharmacol.* 46: 246-255.
3. Yamin, M., et al. 1994. Cloning and characterization of a mouse brain calcitonin receptor complementary deoxyribonucleic acid and mapping of the calcitonin receptor gene. *Endocrinology* 135: 2635-2643.
4. Chen, Y., et al. 1998. The calcitonin receptor stimulates Shc tyrosine phosphorylation and Erk1/2 activation. Involvement of G_i , protein kinase C, and calcium. *J. Biol. Chem.* 273: 19809-19816.
5. Shyu, J.F., et al. 1999. Protein kinase C antagonizes pertussis-toxin-sensitive coupling of the calcitonin receptor to adenylyl cyclase. *Eur. J. Biochem.* 262: 95-101.
6. Nishikawa, T., et al. 1999. A novel calcitonin receptor gene in human osteoclasts from normal bone marrow. *FEBS Lett.* 458: 409-414.

CHROMOSOMAL LOCATION

Genetic locus: Calcr (rat) mapping to 4q13.

PRODUCT

CT-R 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 CT-R shRNA Plasmid (r): sc-270180-SH and CT-R shRNA (r) Lentiviral Particles: sc-270180-V as alternate gene silencing products.

For independent verification of CT-R (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-270180A, sc-270180B and sc-270180C.

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

CT-R siRNA (r) is recommended for the inhibition of CT-R 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CT-R gene expression knockdown using RT-PCR Primer: CT-R (r)-PR: sc-270180-PR (20 μ l). 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 for detailed protocols and support products.