

CALCA siRNA (r): sc-270063

BACKGROUND

Calcitonin is a 32 amino acid polypeptide hormone that preserves skeletal integrity and reduces blood calcium levels by decreasing osteoclast activity in bones, calcium and phosphate reabsorption by kidney tubules and calcium absorption by the intestines. The secretion of Calcitonin from the thyroid is regulated in part by estrogen, which increases Calcitonin mRNA levels. The Calcitonin gene, CALCA, undergoes tissue-specific RNA alternative splicing, resulting in the production of different mRNA transcripts. One transcript encodes procalcitonin as well as both calcium-lowering processed active polypeptides, Calcitonin and katecalcitonin. An alternative transcript of CALCA encodes the precursor for the neuropeptide referred to as Calcitonin gene-related peptide 1, also designated CGRP1 or α -CGRP. CGRP is a widely distributed vasodilatory peptide. Calcitonin and katecalcitonin are produced primarily in the thyroid, while CGRP is produced in neuronal cells. A second CGRP related gene, CALCB, thought to be derived from a gene duplication event, has been identified in mouse, rat and human. Unlike CALCA, CALCB is not subject to alternative splicing and encodes a single transcript designated CGRP2 or β -CGRP. Mature CGRP1 and CGRP2 share significant sequence identity at the protein level differing by only 1-3 amino acid residues, depending on the species.

REFERENCES

1. Le Moullec, J.M., et al. 1984. The complete sequence of human preprocalcitonin. *FEBS Lett.* 167: 93-97.
2. Höppener, J.W., et al. 1985. The second human Calcitonin/CGRP gene is located on chromosome 11. *Hum. Genet.* 70: 259-263.
3. Amara, S.G., et al. 1985. Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to Calcitonin gene-related peptide. *Science* 229: 1094-1097.
4. Wronski, T.J., et al. 1991. Skeletal effects of Calcitonin in ovariectomized rats. *Endocrinology* 129: 2246-2250.
5. Hoovers, J.M., et al. 1993. High-resolution chromosomal localization of the human Calcitonin/CGRP/APP gene family members. *Genomics* 15: 525-529.
6. Silver, J. and Naveh-Many, T. 1993. Calcitonin gene regulation *in vivo*. *Horm. Metab. Res.* 25: 470-472.

CHROMOSOMAL LOCATION

Genetic locus: Calca (rat) mapping to 1q34.

PRODUCT

CALCA siRNA (r) is a pool of 2 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 CALCA shRNA Plasmid (r): sc-270063-SH and CALCA shRNA (r) Lentiviral Particles: sc-270063-V as alternate gene silencing products.

For independent verification of CALCA (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-270063A and sc-270063B.

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

CALCA siRNA (r) is recommended for the inhibition of CALCA 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

CGRP (4901): sc-57053 is recommended as a control antibody for monitoring of CALCA 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 CALCA gene expression knockdown using RT-PCR Primer: CALCA (r)-PR: sc-270063-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.