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CRLR shRNA (m) Lentiviral Particles: sc-44817-V

BACKGROUND

Adrenomedullin (ADM) is a hypotensive peptide that belongs to a peptide superfamily which includes the Calcitonin gene-related peptide (CGRP), a potent vasodilator, and Amylin. Three distinct receptors have the ability to bind ADM and are designated ADM receptor (also designated L1), RDC-1 and the Calcitonin receptor-like receptor (CRLR). The CRLR associates with receptor activity-modifying proteins (RAMPs), which determine the specificity of CRLR binding. Coexpression with RAMP1 results in CRLR binding to CGRP, whereas association with RAMP2 or 3 results in ADM binding. These RAMP proteins mediate the level of glycosylation of CRLR, which in turn, determines the receptors' specificity. CRLR is expressed in heart and blood vessels, which suggests its involvement in vasodilation, smooth muscle relaxation and angiogenesis. RDC-1 is also expressed in heart as well as lung and primarily binds CGRP.

REFERENCES

1. Autelitano, D.J. 1998. Cardiac expression of genes encoding putative adrenomedullin/Calcitonin gene-related peptide receptors. *Biochem. Biophys. Res. Comm.* 250: 689-693.
2. Autelitano, D.J., et al. 1999. Coexpression of prepro-adrenomedullin with a putative adrenomedullin receptor gene in vascular smooth muscle. *Clin. Sci.* 96: 493-498.
3. Mazzocchi, G., et al. 1999. Distribution, functional role, and signaling mechanism of adrenomedullin receptors in the rat adrenal gland. *Peptides* 20: 1479-1487.
4. Ladoux, A., et al. 2000. Coordinated upregulation by hypoxia of adrenomedullin and one of its putative receptors (RDC-1) in cells of the rat blood-brain barrier. *J. Biol. Chem.* 275: 39914-39919.

CHROMOSOMAL LOCATION

Genetic locus: Calcr1 (mouse) mapping to 2 D.

PRODUCT

CRLR shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see CRLR siRNA (m): sc-44817 and CRLR shRNA Plasmid (m): sc-44817-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

CRLR shRNA (m) Lentiviral Particles is recommended for the inhibition of CRLR expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

CRLR (H-42): sc-30028 is recommended as a control antibody for monitoring of CRLR 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CRLR gene expression knockdown using RT-PCR Primer: CRLR (m)-PR: sc-44817-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.