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CART siRNA (r): sc-270029

BACKGROUND

The CART gene encodes for a protein which has an important role in the regulation of appetite and body weight. The CART (cocaine and amphetamine regulated transcript) neuropeptide is an mRNA that changes in response to psychostimulant drug administration. Injection of CART peptides into the ventral tegmental area produces psychostimulant-like effects. CART localizes to areas of the central and peripheral nervous systems and is involved in feeding behavior when injected centrally. Expression of CART in the rat hypothalamus is modulated by nutritional status, and injection of synthetic CART peptide into the forebrain ventricular system suppresses food intake, indicating a possible role in hypothalamic control of energy homeostasis. Its identification in cell bodies and central terminals of vagal afferent neurons additionally suggests a role in brainstem mechanisms of meal termination and satiety.

REFERENCES

1. Dallvechia-Adams, S., et al. 2001. CART peptide-immunoreactive projection from the nucleus accumbens targets substantia nigra pars reticulata neurons in the rat. *J. Comp. Neurol.* 434: 29-39.
2. Kuhar, M.J., et al. 2001. Genes in drug abuse. *Drug Alcohol Depend.* 62: 157-162.
3. Barrett, P., et al. 2001. The differential regulation of CART gene expression in a pituitary cell line and primary cell cultures of ovine pars tuberalis cells. *J. Neuroendocrinol.* 13: 347-352.
4. Cowles, R.A., et al. 2001. Stimulation of rat pancreatic exocrine secretion by cocaine- and amphetamine-regulated transcript peptide. *Regul. Pept.* 99: 61-68.

CHROMOSOMAL LOCATION

Genetic locus: Cartpt (rat) mapping to 2q12.

PRODUCT

CART 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 CART shRNA Plasmid (r): sc-270029-SH and CART shRNA (r) Lentiviral Particles: sc-270029-V as alternate gene silencing products.

For independent verification of CART (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-270029A, sc-270029B and sc-270029C.

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

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

CART (3E4): sc-293241 is recommended as a control antibody for monitoring of CART 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 CART gene expression knockdown using RT-PCR Primer: CART (r)-PR: sc-270029-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Heldsinger, A., et al. 2012. Cocaine- and amphetamine-regulated transcript is the neurotransmitter regulating the action of cholecystokinin and leptin on short-term satiety in rats. *Am. J. Physiol. Gastrointest. Liver Physiol.* 303: G1042-G1051.
2. Li, J.Y., et al. 2019. Altered R-spondin 1/CART neurocircuit in the hypothalamus contributes to hyperphagia in diabetes. *J. Neurophysiol.* 121: 928-939.

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.