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CART shRNA (h) Lentiviral Particles: sc-43664-V

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., Smith, Y. and Kuhar, M.J. 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., Joyce, A. and Dominguez, G. 2001. Genes in drug abuse. *Drug Alcohol Depend.* 62: 157-162.
3. Barrett, P., Morris, M.A., Moar, K.M., Mercer, J.G., Davidson, J.A., Findlay, P.A., Adam, C.L. and Morgan, P.J. 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., Segura, B.J. and Mulholland, M.W. 2001. Stimulation of rat pancreatic exocrine secretion by cocaine- and amphetamine-regulated transcript peptide. *Regul. Pept.* 99: 61-68.

CHROMOSOMAL LOCATION

Genetic locus: CART (human) mapping to 5q13.2.

PRODUCT

CART shRNA (h) 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 CART siRNA (h): sc-43664 and CART shRNA Plasmid (h): sc-43664-SH as alternate gene silencing products.

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.

APPLICATIONS

CART shRNA (h) Lentiviral Particles is recommended for the inhibition of CART expression in human 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

CART (N-20): sc-18068 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CART gene expression knockdown using RT-PCR Primer: CART (h)-PR: sc-43664-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.