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CCK-AR shRNA (h) Lentiviral Particles: sc-43670-V

BACKGROUND

Gastrin is responsible for the stimulation of various digestive functions. In response to Gastrin, the stomach mucosa produces and secretes hydrochloric acid, and the pancreas secretes digestive enzymes. Gastrin also stimulates smooth muscle contraction and increases blood circulation and water secretion in the stomach and intestine. Cholecystokinin (CCK) is a neurotransmitter in the brain that is involved in satiety, stress and anxiety. CCK is expressed in the gastrointestinal (GI) system as well as the central nervous system (CNS), which provides further evidence that CCK modulates food consumption. Both CCK and Gastrin mediate their effects through two G protein-coupled receptors, CCK-AR and CCK-BR. CCK preferentially binds CCK-AR with high affinity, whereas CCK-BR binds to Gastrin and CCK with nearly equal affinities. The cholecystokinin receptors and their ligands are potential therapeutic targets for GI or CNS diseases.

REFERENCES

1. Koh, T.J., et al. 1995. Molecular cloning and sequencing of the murine Gastrin gene. *Biochem. Biophys. Res. Commun.* 216: 34-41.
2. Yassin, R.R. 1999. Signaling pathways mediating Gastrin's growth-promoting effects. *Peptides* 20: 885-898.
3. de Tullio, P., et al. 2000. Therapeutic and chemical developments of cholecystokinin receptor ligands. *Expert Opin. Investig. Drugs* 9: 129-146.
4. Crespi, F., et al. 2000. Involvement of cholecystokinin within craving for cocaine: role of cholecystokinin receptor ligands. *Expert Opin. Investig. Drugs* 9: 2249-2258.
5. Beglinger, C., et al. 2001. Loxiglumide, a CCK-A receptor antagonist, stimulates calorie intake and hunger feelings in humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 280: 1149-1154.

CHROMOSOMAL LOCATION

Genetic locus: CCKAR (human) mapping to 4p15.2.

PRODUCT

CCK-AR 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 CCK-AR siRNA (h): sc-43670 and CCK-AR shRNA Plasmid (h): sc-43670-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

CCK-AR shRNA (h) Lentiviral Particles is recommended for the inhibition of CCK-AR 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

CCK-AR (N-20): sc-16172 is recommended as a control antibody for monitoring of CCK-AR 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 CCK-AR gene expression knockdown using RT-PCR Primer: CCK-AR (h)-PR: sc-43670-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.