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APLNR shRNA (h) Lentiviral Particles: sc-44732-V

BACKGROUND

The apelin receptor (APLNR) is a G protein-coupled integral membrane protein exhibiting a hypothalamic distribution in brain, glial cells, astrocytes and neuronal subpopulations. APLNR is bound by its cognate neuropeptide ligand, apelin, promoting receptor internalization to the nucleus and dose-dependent inhibition of forskolin-induced cAMP production. However, deletion studies of the apelin agonist have shown that internalization is not mandatory for decreasing vasopressin release, a hypotensive action of APLNR signaling. Further evidence for functional dissociation of APLNR stimulation and internalization was exhibited *in vitro* using mutational studies of a nuclear localization signal sequence. These findings may suggest the presence of multiple, functionally-differing conformational states for the receptor. Stress studies in rodents have shown APLNR is under negative regulation by glucocorticoids and may be involved in controlling hypothalamic function. APLNR also functions as an alternate coreceptor with CD4 for HIV-1 infection.

REFERENCES

1. De Mota, N., et al. 2000. Cloning, pharmacological characterization and brain distribution of the rat apelin receptor. *Neuroendocrinology* 72: 400-407.
2. Reaux, A., et al. 2001. Physiological role of a novel neuropeptide, apelin, and its receptor in the rat brain. *J. Neurochem.* 77: 1085-1096.
3. O'Carroll, A.M., et al. 2003. APJ receptor mRNA expression in the rat hypothalamic paraventricular nucleus: regulation by stress and gluco-corticoids. *J. Neuroendocrinol.* 15: 1095-1101.
4. El Messari, S., et al. 2004. Functional dissociation of apelin receptor signaling and endocytosis: implications for the effects of apelin on arterial blood pressure. *J. Neurochem.* 90: 1290-1301.

CHROMOSOMAL LOCATION

Genetic locus: APLNR (human) mapping to 11q12.1.

PRODUCT

APLNR 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 APLNR siRNA (h): sc-44732 and APLNR shRNA Plasmid (h): sc-44732-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

APLNR shRNA (h) Lentiviral Particles is recommended for the inhibition of Apelin Receptor 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

APLNR (H-300): sc-33823 is recommended as a control antibody for monitoring of APLNR 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 Apelin Receptor gene expression knockdown using RT-PCR Primer: APLNR (h)-PR: sc-44732-PR (20 μ l, 550 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.