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# proenkephalin A shRNA (h) Lentiviral Particles: sc-45771-V

## BACKGROUND

The proenkephalin precursor proteins are secreted proteins belonging to the opioid neuropeptide precursor family. The proenkephalin proteins are proteolytically processed to form active secreted opioid peptides, which function as ligands for the  $\kappa$ -type of opioid receptor. The proenkephalin A precursor contains Synenkephalin, Leu-enkephalin and Met-enkephalin processed active peptides, while the proenkephalin B precursor contains  $\beta$ -neoendorphin, Dynorphin, Leu-enkephalin and rimorphin processed active peptides. Proenkephalin A, also designated PENK, preproenkephalin or enkephalin A, is a 267 amino acid secreted protein containing an N-terminal domain which plays a role in disulfide bonding.

## REFERENCES

1. Roberts, J.L., et al. 1979. Corticotropin and  $\beta$ -endorphin: construction and analysis of recombinant DNA complementary to mRNA for the common precursor. *Proc. Natl. Acad. Sci. USA* 76: 2153-2157.
2. Notake, M., et al. 1983. Isolation and characterization of the mouse corticotropin- $\beta$ -lipotropin precursor gene and a related pseudogene. *FEBS Lett.* 156: 67-71.
3. Thorne, B.A., et al. 1989. Expression of mouse proopiomelanocortin in an Insulinoma cell line. Requirements for  $\beta$ -endorphin processing. *J. Biol. Chem.* 264: 3545-3552.
4. Smith, E.M., et al. 1990. Nucleotide and amino acid sequence of lymphocyte-derived corticotropin: endotoxin induction of a truncated peptide. *Proc. Natl. Acad. Sci. USA* 87: 1057-1060.
5. Lee, B.D., et al. 2005. Leu-enkephalin has an anti-apoptotic effect by activating epidermal growth factor receptor kinase in rat pheochromocytoma PC12 cells. *J. Neurochem.* 95: 56-67.
6. Younes, A., et al. 2005. Ischemic preconditioning increases the bioavailability of cardiac enkephalins. *Am. J. Physiol. Heart. Circ. Physiol.* 289: H1652-H1661.

## CHROMOSOMAL LOCATION

Genetic locus: PENK (human) mapping to 8q12.1.

## PRODUCT

proenkephalin A 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  $\mu$ l frozen stock containing  $1.0 \times 10^6$  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 proenkephalin A siRNA (h): sc-45771 and proenkephalin A shRNA Plasmid (h): sc-45771-SH as alternate gene silencing products.

## STORAGE

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

## APPLICATIONS

proenkephalin A shRNA (h) Lentiviral Particles is recommended for the inhibition of proenkephalin A expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  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

Met/Leu-enkephalin (NOC1/35): sc-47705 is recommended as a control antibody for monitoring of proenkephalin A 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-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (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-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor proenkephalin A gene expression knockdown using RT-PCR Primer: proenkephalin A (h)-PR: sc-45771-PR (20  $\mu$ 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.

## PROTOCOLS

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