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CPE shRNA (m) Lentiviral Particles: sc-45379-V

BACKGROUND

Carboxypeptidase N (arginine carboxypeptidase or CPN) cleaves basic amino acid residues from the C-terminus of peptides and proteins. The enzyme plays a central role in regulating the biologic activity of peptides such as kinins and anaphylatoxins, and therefore is also known as kininase-1 and anaphylatoxin inactivator. CPN is a tetrameric complex consisting of two identical regulatory subunits (CPN reg) and two identical catalytic subunits (CPN cat). CPN reg is a member of the leucine-rich repeat family of proteins and CPN cat is a member of the regulatory B-type carboxypeptidase group. Carboxypeptidase E (CPE) is important for removing any remaining C-terminal Arg or Lys after initial endoprotease cleavage during prohormone processing. CPE is also crucial in proinsulin processing, and required for normal-sized photoreceptor synaptic terminal and normal signal transmission to the inner retina.

REFERENCES

- Zhu, X., et al. 2005. Carboxypeptidase E is required for normal synaptic transmission from photoreceptors to the inner retina. *J. Neurochem.* 95: 1351-1362.
- Hosaka, M., et al. 2005. Interaction between secretogranin III and carboxypeptidase E facilitates prohormone sorting within secretory granules. *J. Cell. Sci.* 118: 4785-4795.
- Johnston, R.A., et al. 2005. Augmented responses to ozone in obese carboxypeptidase E deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290: R126-133.
- Marzban, L., et al. 2005. Role of carboxypeptidase E in processing of pro-islet amyloid polypeptide in β cells. *Endocrinology* 146: 1808-1817.
- Lou, H., et al. 2005. Sorting and activity-dependent secretion of BDNF require interaction of a specific motif with the sorting receptor carboxypeptidase E. *Neuron* 45: 245-255.

CHROMOSOMAL LOCATION

Genetic locus: *Cpe* (mouse) mapping to 8 B3.1.

PRODUCT

CPE shRNA (m) 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 CPE siRNA (m): sc-45379 and CPE shRNA Plasmid (m): sc-45379-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

CPE shRNA (m) Lentiviral Particles is recommended for the inhibition of CPE expression in mouse 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

CPE (H-5): sc-393761 is recommended as a control antibody for monitoring of CPE 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 CPE gene expression knockdown using RT-PCR Primer: CPE (m)-PR: sc-45379-PR (20 μ l). 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.