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CPE siRNA (h): sc-45378

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

1. Zhu, X., et al. 2005. Carboxypeptidase E is required for normal synaptic transmission from photoreceptors to the inner retina. *J. Neurochem.* 95: 1351-1362.
2. Hosaka, M., et al. 2005. Interaction between secretogranin III and carboxypeptidase E facilitates prohormone sorting within secretory granules. *J. Cell Sci.* 118: 4785-4795.
3. Johnston, RA., et al. 2005. Augmented responses to ozone in obese carboxypeptidase E deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290: R126-R133.
4. Marzban, L., et al. 2005. Role of carboxypeptidase E in processing of pro-islet amyloid polypeptide in β cells. *Endocrinology* 146: 1808-1817.
5. 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 (human) mapping to 4q32.3.

PRODUCT

CPE siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CPE shRNA Plasmid (h): sc-45378-SH and CPE shRNA (h) Lentiviral Particles: sc-45378-V as alternate gene silencing products.

For independent verification of CPE (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45378A, sc-45378B and sc-45378C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CPE siRNA (h) is recommended for the inhibition of CPE expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

CPE (H-6): sc-393760 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CPE gene expression knockdown using RT-PCR Primer: CPE (h)-PR: sc-45378-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.