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CCP6 siRNA (m): sc-142171

BACKGROUND

The peptidase M14 family of carboxypeptidases (CPs) are involved in various functions throughout the body which include digestion of food and biosynthesis of peptides that function in intercellular signaling. CCP6 (Cytosolic carboxypeptidase 6), also known as AGBL4 (ATP/GTP binding protein-like 4), is a 540 amino acid cytoplasmic protein that is expressed in testis, pituitary and brain, with more moderate expression observed in eye, stomach, kidney and adrenal tissue. It is suggested that CCP6 may be involved in processing of cytosolic proteins such as α Tubulin. The gene encoding CCP6 maps to human chromosome 1p33, the largest human chromosome spanning about 260 million base pairs and making up 8% of the human genome. There are about 3,000 genes on chromosome 1, and considering the great number of genes there are also a large number of diseases associated with chromosome 1 which include Hutchinson-Gilford progeria, familial adenomatous polyposis, Parkinsons, Gaucher disease and Usher syndrome.

REFERENCES

1. Harris, A., et al. 2000. Regenerating motor neurons express Nna1, a novel ATP/GTP-binding protein related to zinc carboxypeptidases. *Mol. Cell. Neurosci.* 16: 578-596.
2. Weise, A., et al. 2005. New insights into the evolution of chromosome 1. *Cytogenet. Genome Res.* 108: 217-222.
3. Gregory, S.G., et al. 2006. The DNA sequence and biological annotation of human chromosome 1. *Nature* 441: 315-321.
4. Kalinina, E., et al. 2007. A novel subfamily of mouse cytosolic carboxypeptidases. *FASEB J.* 21: 836-850.
5. Rodriguez de la Vega, M., et al. 2007. Nna1-like proteins are active metal-localcarboxypeptidases of a new and diverse M14 subfamily. *FASEB J.* 21: 851-865.

CHROMOSOMAL LOCATION

Genetic locus: Agbl4 (mouse) mapping to 4 C7.

PRODUCT

CCP6 siRNA (m) 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 CCP6 shRNA Plasmid (m): sc-142171-SH and CCP6 shRNA (m) Lentiviral Particles: sc-142171-V as alternate gene silencing products.

For independent verification of CCP6 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-142171A, sc-142171B and sc-142171C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

CCP6 siRNA (m) is recommended for the inhibition of CCP6 expression in mouse 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

CCP6 (N-14): sc-138893 is recommended as a control antibody for monitoring of CCP6 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 CCP6 gene expression knockdown using RT-PCR Primer: CCP6 (m)-PR: sc-142171-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.