

AGTPBP1 siRNA (h): sc-92665

BACKGROUND

AGTPBP1 (ATP/GTP-binding protein 1), also known as CCP1 (cytosolic carboxypeptidase 1), KIAA1035 or Nna1, is a 1,226 amino acid protein that belongs to the peptidase M14 family. AGTPBP1 contains an ATP/GTP-binding motif of the P-loop type, a leucine zipper, a nuclear localization signal, a zinc carboxypeptidase signature and a nucleotide-binding site. Expressed at high levels in testis, heart and dorsal root ganglia, and at lower levels in skeletal muscle and kidney, AGTPBP1 may be responsible for Purkinje cell degeneration (pcd). The loss of AGTPBP1 in Purkinje cells leads directly to their degeneration and a functional carboxypeptidase domain is crucial for AGTPBP1 to support neuron survival. Two isoforms exist due to alternative splicing events.

REFERENCES

- 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.
- Fernandez-Gonzalez, A., et al. 2002. Purkinje cell degeneration (pcd) phenotypes caused by mutations in the axotomy-induced gene, Nna1. *Science* 295: 1904-1906.
- Delis, F., et al. 2004. Dopamine receptor and transporter levels are altered in the brain of Purkinje cell degeneration mutant mice. *Neuroscience* 125: 255-268.
- Chakrabarti, L., et al. 2006. The Purkinje cell degeneration 5J mutation is a single amino acid insertion that destabilizes Nna1 protein. *Mamm. Genome* 17: 103-110.
- Wang, T., et al. 2006. The carboxypeptidase-like substrate-binding site in Nna1 is essential for the rescue of the Purkinje cell degeneration (pcd) phenotype. *Mol. Cell. Neurosci.* 33: 200-213.

CHROMOSOMAL LOCATION

Genetic locus: AGTPBP1 (human) mapping to 9q21.33.

PRODUCT

AGTPBP1 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 AGTPBP1 shRNA Plasmid (h): sc-92665-SH and AGTPBP1 shRNA (h) Lentiviral Particles: sc-92665-V as alternate gene silencing products.

For independent verification of AGTPBP1 (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-92665A, sc-92665B and sc-92665C.

RESEARCH USE

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

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

AGTPBP1 siRNA (h) is recommended for the inhibition of AGTPBP1 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 contains 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

AGTPBP1 (LM-1A7): sc-134251 is recommended as a control antibody for monitoring of AGTPBP1 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 AGTPBP1 gene expression knockdown using RT-PCR Primer: AGTPBP1 (h)-PR: sc-92665-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.