SANTA CRUZ BIOTECHNOLOGY, INC.

PNAd siRNA (m): sc-62833

BACKGROUND

PNAd, protein N-terminal asparagine amidohydrolase, is a 310 amino acid protein encoded by the human gene NTAN1. PNAd is responsible for the side-chain deamidation of N-terminal asparagine residues to aspartate. It is required for the ubiquitin-dependent turnover of intracellular proteins that initiate with Met-Asn. These proteins are acetylated on the retained initiator methionine and can subsequently be modified by the removal of N-acetyl methionine by acylaminoacid hydrolase (AAH). Conversion of the resulting N-terminal asparagine to aspartate by PNAd renders the protein susceptible to arginylation, polyubiquitination and degradation as specified by the N-end rule. This enzyme does not act on substrates with internal or C-terminal asparagines and does not act on glutamine residues in any position.

REFERENCES

1. Grigoryev, S., et al. 1996. A mouse amidase specific for N-terminal asparagine. The gene, the enzyme, and their function in the N-end rule pathway. J. Biol. Chem. 271: 28521-28532.
2. Balogh, S.A., et al. 2000. Varying intertrial interval reveals temporally defined memory deficits and enhancements in NTAN1-deficient mice. Learn. Mem. 7: 279-286.
3. Kwon, Y.T., et al. 2000. Altered activity, social behavior, and spatial memory in mice lacking the NTAN1p amidase and the asparagine branch of the N-end rule pathway. Mol. Cell. Biol. 20: 4135-4148.
4. Balogh, S.A., et al. 2001. Facilitated stimulus-response associative learning and long-term memory in mice lacking the NTAN1 amidase of the N-end rule pathway. Brain Res. 892: 336-343.
5. Balogh, S.A., et al. 2003. Behavioral characterization of mice lacking the ubiquitin ligase UBR1 of the N-end rule pathway. Genes Brain Behav. 1: 223-229.
6. Goto, Y., et al. 2006. The magnetism responsive gene Ntan1 in mouse brain. Neurochem. Int. 49: 334-341.
7. Hirai, T., et al. 2006. Stimulation of ubiquitin-proteasome pathway through the expression of amidohydrolase for N-terminal asparagine (Ntan1) in cultured rat hippocampal neurons exposed to static magnetism. J. Neurochem. 96: 1519-1530.

CHROMOSOMAL LOCATION

Genetic locus: Ntan1 (mouse) mapping to 16 A1.

PRODUCT

PNAd 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PNAd shRNA Plasmid (m): sc-62833-SH and PNAd shRNA (m) Lentiviral Particles: sc-62833-V as alternate gene silencing products.

For independent verification of PNAd (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-62833A, sc-62833B and sc-62833C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PNAd siRNA (m) is recommended for the inhibition of PNAd 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 µM in 66 µl. 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

PNAd (E-11): sc-166493 is recommended as a control antibody for monitoring of PNAd 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 PNAd gene expression knockdown using RT-PCR Primer: PNAd (m)-PR: sc-62833-PR (20 µl). 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.

PROTOCOLS

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