SANTA CRUZ BIOTECHNOLOGY, INC.

BNPI siRNA (m): sc-29817

BACKGROUND

The BNPI (brain specific Na^+-dependent inorganic phosphate(Pi) cotransporter) gene, also designated VGLUT1 (vesicular glutamate transporter), is located on chromosome 19q13.33 and encodes a 560 amino acid protein with 6-8 transmembrane-spanning domains. BNPI is expressed predominantly in neurons of the cerebral cortex, hippocampus, and cerebellum, and is more highly expressed in adult brain compared to fetal brain. BNPI localizes almost exclusively to nerve terminals forming asymmetric excitatory-type synapses and associates preferentially with the membranes of small synaptic vesicles. In the plasma membrane, BNP1 imports phospate ions, which are required for glutmatergic neurotransmission. Expression of BNPI results in glutamate uptake by intracellular vesicles, which defines a glutamatergic phenotype in neurons.

REFERENCES

1. Ni, B., et al. 1994. Cloning and expression of a CDNA encoding a brain-specific Na^+-dependent inorganic phosphate cotransporter. Proc. Natl. Acad. Sci. USA 91: 5607-5611.
2. Ni, B., et al. 1996. Molecular cloning, expression, and chromosomal localization of a human brain-specific Na^+-dependent inorganic phospate cotransporter. J. Neurochem. 66: 227-238.
3. Bellocchio, E.E., et al. 1998. The localization of the brain-specific inorganic phosphate transporter suggests a specific presynaptic role in glutamatergic transmission. J. Nuerosci. 18: 8648-8659.
4. Lee, R.Y., et al. 1999. EAT-4, a homolog of a mammalian sodium dependent inorganic phosphate cotransporter, is necessary for glutamatergic neurotransmission in *Caenorhabditis elegans*. J. Neurosci. 19: 159-167.
5. Bellocchio, E.E., et al. 2000. Uptake of glutamate into synaptic vesicles by an inorganic phosphate transporter. Science 289: 957-960.
6. Takamori, S., et al. 2000. Identification of a vesicular glutamate transporter that defines a glutamatergic phenotype in neurons. Nature 407: 189-194.

CHROMOSOMAL LOCATION

Genetic locus: Slc17a7 (mouse) mapping to 7 B4.

PRODUCT

BNPI 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 BNPI shRNA Plasmid (m): sc-29817-SH and BNPI shRNA (m) Lentiviral Particles: sc-29817-V as alternate gene silencing products.

For independent verification of BNPI (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-29817A, sc-29817B and sc-29817C.

RESEARCH USE

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

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

BNPI siRNA (m) is recommended for the inhibition of BNPI 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

BNPI (A-8): sc-377425 is recommended as a control antibody for monitoring of BNPI 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 BNPI gene expression knockdown using RT-PCR Primer: BNPI (m)-PR: sc-29817-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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