SANTA CRUZ BIOTECHNOLOGY, INC.

DNPEP siRNA (h): sc-95014

BACKGROUND

DNPEP, also known as DAP, ASPEP or aspartyl aminopeptidase, is a 475 amino acid protein that is a member of the M18 family of the MH clan of co-catalytic metallopeptidases. It contains three zinc finger binding domains and several conserved residues including three histidines, three glutamates and five aspartates. DNPEP is ubiquitously expressed with highest expression in testis, intermediate expression in kidney and lung, and lesser but significant expression in spleen, liver and brain. DNPEP removes glutamyl or aspartyl residues from N-terminal acidic amino acid-containing peptides, implicating its importance in intracellular protein and peptide metabolism. In the brain, DNPEP converts Angiotensin I to Angiotensin II and is thought to play an important role in blood pressure control. This suggests that DNPEP may function as a potential target for antihypertensive therapy.

REFERENCES

1. Wilk, S., et al. 1998. Purification, characterization, and cloning of a cytosolic aspartyl aminopeptidase. J. Biol. Chem. 273: 15961-15970.
2. Ramírez-Expósito, M.J., et al. 2000. Comparative distribution of glutamyl and aspartyl aminopeptidase activities in mouse organs. Horm. Metab. Res. 32: 161-163.
3. Wilk, S., et al. 2002. Identification of histidine residues important in the catalysis and structure of aspartyl aminopeptidase. Arch. Biochem. Biophys. 407: 176-183.
4. Varona, A., et al. 2003. Effects of changes in hydromineral balance on rat brain aspartyl, arginyl, and alanyl aminopeptidase activities. Horm. Metab. Res. 35: 36-42.
5. Banegas, I., et al. 2006. Brain aminopeptidases and hypertension. J. Renin Angiotensin Aldosterone Syst. 7: 129-134.
6. Carrera, M.P., et al. 2006. Renin-angiotensin system-regulating aminopeptidase activities are modified in the pineal gland of rats with breast cancer induced by N-methyl-nitrosourea. Cancer Invest. 24: 149-153.
7. Yokoyama, R., et al. 2006. Identification of yeast aspartyl aminopeptidase gene by purifying and characterizing its product from yeast cells. FEBS J. 273: 192-198.

CHROMOSOMAL LOCATION

Genetic locus: DNPEP (human) mapping to 2q35.

PRODUCT

DNPEP 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DNPEP shRNA Plasmid (h): sc-95014-SH and DNPEP shRNA (h) Lentiviral Particles: sc-95014-V as alternate gene silencing products.

For independent verification of DNPEP (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-95014A, sc-95014B and sc-95014C.

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

DNPEP siRNA (h) is recommended for the inhibition of DNPEP 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 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

DNPEP (Q-16): sc-100492 is recommended as a control antibody for monitoring of DNPEP 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 DNPEP gene expression knockdown using RT-PCR Primer: DNPEP (h)-PR: sc-95014-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.