SANTA CRUZ BIOTECHNOLOGY, INC.

Aminopeptidase P1 siRNA (m): sc-141046

BACKGROUND

Aminopeptidases comprise a family of enzymatic proteins that are widely distributed in both eukaryotes and prokaryotes and function to catalyze the removal of amino acids from the N-termini of proteins. Aminopeptidase P1 is proline-specific; it cleaves the N-terminal amino acid where the second residue is proline. It is a mammalian bradykinin-degrading, metal-dependant enzyme that exists in two forms: a membrane-bound form and a cytosolic form. Aminopeptidase P1 is GPI-linked and the membrane-bound form is expressed in all tissues with highest expression in pancreas. Aminopeptidase P1 has been shown to be a receptor for the breast-homing peptide and may therefore be a potential therapeutic target for the treatment and prevention of breast cancer.

REFERENCES

1. Wilk, S., et al. 1998. Purification, characterization and cloning of a cytosolic aspartyl aminopeptidase. J. Biol. Chem. 273: 15961-15970.
2. Cottrell, G.S., et al. 2000. Cloning, expression and characterization of human cytosolic Aminopeptidase P: a single manganese(II)-dependent enzyme. Biochemistry 39: 15121-15128.
3. Essler, M., et al. 2002. Molecular specialization of breast vasculature: a breast-homing phage-displayed peptide binds to Aminopeptidase P in breast vasculature. Proc. Natl. Acad. Sci. USA 99: 2252-2257.
4. Graham, S.C., et al. 2006. Kinetic and crystallographic analysis of mutant *Escherichia coli* Aminopeptidase P: insights into substrate recognition and the mechanism of catalysis. Biochemistry 45: 964-975.
5. Jao, S.C., et al. 2006. Tyrosine 387 and Arginine 404 are critical in the hydrolytic mechanism of *Escherichia coli* Aminopeptidase P. Biochemistry 45: 1547-1553.
6. Kiraly, O., et al. 2006. Expression of human cationic trypsinogen with an authentic N-terminus using intein-mediated splicing in Aminopeptidase P deficient *Escherichia coli*. Protein Expr. Purif. 48: 104-111.
7. Molinaro, G., et al. 2006. Kinin-dependent hypersensitivity reactions in hemodialysis: metabolic and genetic factors. Kidney Int. 70: 1823-1831.

CHROMOSOMAL LOCATION

Genetic locus: Xpnpep1 (mouse) mapping to 19 D2.

PRODUCT

Aminopeptidase P1 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 Aminopeptidase P1 shRNA Plasmid (m): sc-141046-SH and Aminopeptidase P1 shRNA (m) Lentiviral Particles: sc-141046-V as alternate gene silencing products.

For independent verification of Aminopeptidase P1 (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-141046A, sc-141046B and sc-141046C.

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

Aminopeptidase P1 siRNA (m) is recommended for the inhibition of Aminopeptidase P1 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

Aminopeptidase P1 (H-4): sc-514272 is recommended as a control antibody for monitoring of Aminopeptidase P1 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 Aminopeptidase P1 gene expression knockdown using RT-PCR Primer: Aminopeptidase P1 (m)-PR: sc-141046-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.