

PAT4 siRNA (h): sc-96627

BACKGROUND

The proton-coupled amino acid transporter family consists of four family members, namely PAT1, PAT2, PAT3 and PAT4, all of which mediate the 1:1 symport of protons and small neutral amino acids and derivatives across both intracellular and plasma membranes. Substrates for the PAT family members include L- and D-proline, glycine and L-alanine, 3-amino-1-propanesulfonic acid, L-azetidine-2-carboxylic acid and cis-4-hydroxy-D-proline. PAT1 expression is high in intestine and brain where it localizes to the brush border membrane, thereby allowing PAT1 to serve as a novel route for oral drug delivery. PAT2 shows high expression in spinal cord and brain, while PAT3 expression is found in testis. PAT4 is a multi-pass membrane protein that is expressed as two alternatively spliced isoforms. All four PAT family members contain three conserved histidine residues with His-55 found to be essential for catalytic activity of PAT1.

REFERENCES

1. Boll, M., Foltz, M., Rubio-Aliaga, I. and Daniel, H. 2003. A cluster of proton/amino acid transporter genes in the human and mouse genomes. *Genomics* 82: 47-56.
2. Foltz, M., Oechsler, C., Boll, M., Kottra, G. and Daniel, H. 2004. Substrate specificity and transport mode of the proton-dependent amino acid transporter mPAT2. *Eur. J. Biochem.* 271: 3340-3347.
3. Rubio-Aliaga, I., Boll, M., Vogt Weisenhorn, D.M., Foltz, M., Kottra, G. and Daniel, H. 2004. The proton/amino acid cotransporter PAT2 is expressed in neurons with a different subcellular localization than its paralog PAT1. *J. Biol. Chem.* 279: 2754-2760.
4. Boll, M., Daniel, H. and Gasnier, B. 2004. The SLC36 family: proton-coupled transporters for the absorption of selected amino acids from extracellular and intracellular proteolysis. *Pflugers Arch.* 447: 776-779.
5. Metzner, L., Neubert, K. and Brandsch, M. 2006. Substrate specificity of the amino acid transporter PAT1. *Amino Acids* 31: 111-117.
6. Metzner, L. and Brandsch, M. 2006. Influence of a proton gradient on the transport kinetics of the H⁺/amino acid cotransporter PAT1 in Caco-2 cells. *Eur J Pharm Biopharm.* 63: 360-364.
7. Thwaites, D.T. and Anderson, C.M. 2007. Deciphering the mechanisms of intestinal imino (and amino) acid transport: the redemption of SLC36A1. *Biochim. Biophys. Acta* 1768: 179-197.
8. Metzner, L., Natho, K., Zebisch, K., Dorn, M., Bosse-Doenecke, E., Ganapathy, V. and Brandsch, M. 2008. Mutational analysis of histidine residues in the human proton-coupled amino acid transporter PAT1. *Biochim. Biophys. Acta* 1778: 1042-1050.

CHROMOSOMAL LOCATION

Genetic locus: SLC36A4 (human) mapping to 11q21.

PROTOCOLS

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

PRODUCT

PAT4 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 PAT4 shRNA Plasmid (h): sc-96627-SH and PAT4 shRNA (h) Lentiviral Particles: sc-96627-V as alternate gene silencing products.

For independent verification of PAT4 (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-96627A, sc-96627B and sc-96627C.

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

PAT4 siRNA (h) is recommended for the inhibition of PAT4 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PAT4 gene expression knockdown using RT-PCR Primer: PAT4 (h)-PR: sc-96627-PR (20 μ l). 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.