

PAT2 siRNA (h): sc-91668

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

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2. Foltz, M., et al. 2004. Substrate specificity and transport mode of the proton-dependent amino acid transporter mPAT2. *Eur. J. Biochem.* 271: 3340-3347.
3. Rubio-Aliaga, I., et al. 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., et al. 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., et al. 2006. Substrate specificity of the amino acid transporter PAT1. *Amino Acids* 31: 111-117.
6. Metzner, L., et al. 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., et al. 2007. Deciphering the mechanisms of intestinal imino (and amino) acid transport: the redemption of SLC36A1. *Biochim. Biophys. Acta* 1768: 179-197.

CHROMOSOMAL LOCATION

Genetic locus: SLC36A2 (human) mapping to 5q33.1.

PRODUCT

PAT2 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 PAT2 shRNA Plasmid (h): sc-91668-SH and PAT2 shRNA (h) Lentiviral Particles: sc-91668-V as alternate gene silencing products.

For independent verification of PAT2 (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-91668A, sc-91668B and sc-91668C.

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

PAT2 siRNA (h) is recommended for the inhibition of PAT2 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.

GENE EXPRESSION MONITORING

PAT2 (F-3): sc-390969 is recommended as a control antibody for monitoring of PAT2 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PAT2 gene expression knockdown using RT-PCR Primer: PAT2 (h)-PR: sc-91668-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.

PROTOCOLS

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