SANTA CRUZ BIOTECHNOLOGY, INC.

LAT2 siRNA (h): sc-105609

BACKGROUND

L-amino acid transporter protein-2 (LAT2), a non-glycosylated membrane protein, complexes with CD98 to contribute to readsorption of neutral amino acids in renal epithelia and blood-tissue barriers. The gene encoding LAT2 is expressed primarily in the kidney, but also to a lesser extent in placenta, brain, liver, spleen, skeletal muscle, heart, small intestine, and lung. Transfection with the antisense sequence of LAT2 suggests that LAT2 expression plays a major role in net basolateral efflux of cysteine, and points to LAT2 as a candidate gene to modulate cysteine reabsorption. In addition, the CD98/LAT2 heterodimer associates with Integrin β1 in intestinal epithelial cells, where ligand binding to CD98 and another cell surface molecule, ICAM-1 differentially regulates LAT2 activity, suggesting a novel mechanism by which events like cell adhesion may affect amino acid transport activity.

REFERENCES

1. Pineda, M., et al. 1999. Identification of a membrane protein, LAT-2, that Co-expresses with 4F2 heavy chain, an L-type amino acid transport activity with broad specificity for small and large zwitterionic amino acids. J. Biol. Chem. 274: 19738-19744.
2. Segawa, H., et al. 1999. Identification and functional characterization of a Na^+-independent neutral amino acid transporter with broad substrate selectivity. J. Biol. Chem. 274: 19745-19751.
3. Fernandez, E., et al. 2003. Basolateral LAT-2 has a major role in the transepithelial flux of L-cystine in the renal proximal tubule cell line OK. J. Am. Soc. Nephrol. 14: 837-847.
4. Liu, X., et al. 2003. CD98 and intracellular adhesion molecule I regulate the activity of amino acid transporter LAT-2 in polarized intestinal epithelia. J. Biol. Chem. 278: 23672-23677.
5. Pinho, M.J., et al. 2003. Organ-specific overexpression of renal LAT2 and enhanced tubular L-DOPA uptake precede the onset of hypertension. Hypertension 42: 613-618.
6. Kim do, K., et al. 2004. System L-amino acid transporters are differently expressed in rat astrocyte and C6 glioma cells. Neurosci. Res. 50: 437-446.

CHROMOSOMAL LOCATION

Genetic locus: SLC7A8 (human) mapping to 14q11.2.

PRODUCT

LAT2 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 LAT2 shRNA Plasmid (h): sc-105609-SH and LAT2 shRNA (h) Lentiviral Particles: sc-105609-V as alternate gene silencing products.

For independent verification of LAT2 (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-105609A, sc-105609B and sc-105609C.

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

LAT2 siRNA (h) is recommended for the inhibition of LAT2 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

LAT2 (3F10): sc-293242 is recommended as a control antibody for monitoring of LAT2 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 LAT2 gene expression knockdown using RT-PCR Primer: LAT2 (h)-PR: sc-105609-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.