SANTA CRUZ BIOTECHNOLOGY, INC.

LAT1 siRNA (m): sc-62556

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The Power to Question

BACKGROUND

L-type amino acid transporter 1 (LAT1) is a multipass-membrane protein responsible for sodium-independent, high-affinity transport of large neutral amino acids. LAT1 functions as a disulfide-linked heterodimer with the amino acid transport protein CD98. LAT1 is expressed predominantly in adult lung and liver but is also expressed in brain, thymus, retina, testis, placenta, bone marrow and fetal liver. In the retina, LAT1 localizes to the blood-retinal-barrier (BRB) and mediates L-leucine transport from the blood to the retina. The devastating effects on the brain caused by phenylketonuria are due to the increased levels of LAT1 on the blood-brain-barrier in response to high concentrations of phenylalanine in the blood. LAT1 accepts the amino-acid related anticancer agent melphalan and plays a significant role in cell proliferation, differentiation and invasion in esophageal squamous cell carcinoma.

REFERENCES

1. Satoh, S., et al. 1997. Involvement of L-type-like amino acid transporters in S-nitrosocysteine-stimulated noradrenaline release in the rat hippocampus. J. Neurochem. 69: 2197-2205.
2. Yanagida, O., et al. 2001. Human L-type amino acid transporter 1 (LAT1): characterization of function and expression in tumor cell lines. Biochim. Biophys. Acta 1514: 291-302.
3. Tamai, S., et al. 2001. Expression of L-type amino acid transporter 1 in a rat model of liver metastasis: positive correlation with tumor size. Cancer Detect. Prev. 25: 439-445.
4. Ohkame, H., et al. 2001. Expression of L-type amino acid transporter 1 (LAT1) and 4F2 heavy chain (4F2hc) in liver tumor lesions of rat models. J. Surg. Oncol. 78: 265-271.
5. Uchino, H., et al. 2002. Transport of amino acid-related compounds mediated by L-type amino acid transporter 1 (LAT1): insights into the mechanisms of substrate recognition. Mol. Pharmacol. 61: 729-737.

CHROMOSOMAL LOCATION

Genetic locus: Slc7a5 (mouse) mapping to 8 E1.

PRODUCT

LAT1 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 LAT1 shRNA Plasmid (m): sc-62556-SH and LAT1 shRNA (m) Lentiviral Particles: sc-62556-V as alternate gene silencing products.

For independent verification of LAT1 (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-62556A, sc-62556B and sc-62556C.

PROTOCOLS

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

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

LAT1 siRNA (m) is recommended for the inhibition of LAT1 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

LAT1 (D-10): sc-374232 is recommended as a control antibody for monitoring of LAT1 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 LAT1 gene expression knockdown using RT-PCR Primer: LAT1 (m)-PR: sc-62556-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.