

ASCT1 siRNA (h): sc-60208

BACKGROUND

Neutral amino acid transporter proteins, also designated alanine/serine/cysteine/threonine transporters (ASCT), belong to the sodium:dicarboxylate (SDF) symporter family of proteins. The members of this family of proteins are multi-pass, membrane-bound proteins that act as transporters for threonine, alanine, serine and cysteine. ASCT1 and ASCT2 have been shown to exhibit sodium dependence. ASCT1 is expressed in most tissues, but highest expression has been detected in muscle, brain and pancreas. The highest levels of ASCT2 expression are found in placenta, kidney, pancreas, muscle and intestine.

REFERENCES

1. Arriza, J.L., et al. 1993. Cloning and expression of a human neutral amino acid transporter with structural similarity to the glutamate transporter gene family. *J. Biol. Chem.* 268: 15329-15332.
2. Hofmann, K., et al. 1995. Human neutral amino acid transporter ASCT1: structure of the gene (SLC1A4) and localization to chromosome 2p13-p15. *Genomics* 24: 20-26.
3. Kekuda, R., et al. 1996. Cloning of the sodium-dependent, broad-scope, neutral amino acid transporter Bo from a human placental choriocarcinoma cell line. *J. Biol. Chem.* 271: 18657-18661.
4. Rasko, J.E., et al. 1999. The RD114/simian type D retrovirus receptor is a neutral amino acid transporter. *Proc. Natl. Acad. Sci. USA* 96: 2129-2134.
5. Tailor, C.S., et al. 1999. A sodium-depend and baboon endogenous retroviruses and simian type D retroviruses. *J. Virol.* 73: 4470-4474.
6. Tailor, C.S., et al. 2001. Truncated forms of the dual function human ASCT2 neutral amino acid transporter/retroviral receptor are translationally initiated at multiple alternative CUG and GUG codons. *J. Biol. Chem.* 276: 27221-27230.
7. Yamamoto, T., et al. 2004. Functional identification of ASCT1 neutral amino acid transporter as the predominant system for the uptake of L-serine in rat neurons in primary culture. *Neurosci. Res.* 49: 101-111.
8. Kanai, Y. and Hediger, M.A. 2004. The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects. *Pflugers Arch.* 447: 469-479.

CHROMOSOMAL LOCATION

Genetic locus: SLC1A4 (human) mapping to 2p14.

PRODUCT

ASCT1 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 ASCT1 shRNA Plasmid (h): sc-60208-SH and ASCT1 shRNA (h) Lentiviral Particles: sc-60208-V as alternate gene silencing products.

For independent verification of ASCT1 (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-60208A, sc-60208B and sc-60208C.

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

ASCT1 siRNA (h) is recommended for the inhibition of ASCT1 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

ASCT1 (C-8): sc-393157 is recommended as a control antibody for monitoring of ASCT1 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 ASCT1 gene expression knockdown using RT-PCR Primer: ASCT1 (h)-PR: sc-60208-PR (20 μ l, 455 bp). 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.