SANTA CRUZ BIOTECHNOLOGY, INC.

ENT1 siRNA (r): sc-270325

BACKGROUND

Equilibrative nucleoside transporters (ENTs) regulate many physiological processes and are widely distributed in mammals, plants, yeasts, insects, nematodes and protozoans. They enable facilitated diffusion of hydrophilic nucleosides, such as adenosine and nucleoside analogs, across cell membranes. ENTs are required for uptake of antiviral and anticancer nucleoside drugs and influence a variety of physiological processes, such as neurotransmission and platelet aggregation, by regulating the amount of adenoside available to cell surface receptors. Equilibrative nucleoside transporter 1 (ENT1), also designated solute carrier family 29 (nucleoside transporters), member 1, belongs to the SLC29A transporter family and is a mammalian ENT isoform. ENT1, along with ENT3, mediates the majority of influx and efflux of nucleosides across the plasma membrane.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 602193. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Mangravite, L.M., et al. 2003. Localization of human equilibrative nucleoside transporters, hENT1 and hENT2, in renal epithelial cells. Am. J. Physiol. Renal Physiol. 284: 902-910.
3. Vickers, M.F., et al. 2004. Uridine recognition motifs of human equilibrative nucleoside transporters 1 and 2 produced in *Saccharomyces cerevisiae*. Nucleosides Nucleotides Nucleic Acids 23: 361-373.
4. Stolk, M., et al. 2005. Subtype-specific regulation of equilibrative nucleoside transporters by protein kinase CK2. Biochem. J. 386: 281-289.
5. Visser, F., et al. 2005. Identification and mutational analysis of amino acid residues involved in dipyridamole interactions with human and *Caenorhabditis elegans* equilibrative nucleoside transporters. J. Biol. Chem. 280: 11025-11034.
6. Visser, F., et al. 2005. Residue 33 of human equilibrative nucleoside transporter 2 is a functionally important component of both the dipyridamole and nucleoside binding sites. Mol. Pharmacol. 67: 1291-1298.

CHROMOSOMAL LOCATION

Genetic locus: Slc29a1 (rat) mapping to 9q12.

PRODUCT

ENT1 siRNA (r) 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 ENT1 shRNA Plasmid (r): sc-270325-SH and ENT1 shRNA (r) Lentiviral Particles: sc-270325-V as alternate gene silencing products.

For independent verification of ENT1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270325A, sc-270325B and sc-270325C.

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

ENT1 siRNA (r) is recommended for the inhibition of ENT1 expression in rat 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

ENT1 (F-12): sc-377283 is recommended as a control antibody for monitoring of ENT1 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 ENT1 gene expression knockdown using RT-PCR Primer: ENT1 (r)-PR: sc-270325-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.