



ENT4 siRNA (h): sc-89525

BACKGROUND

ENT4 (equilibrative nucleoside transporter 4), also known as SLC29A4 (solute carrier family 29 member 4) or PMAT, is a 530 amino acid plasma membrane protein that is involved in the transport of various compounds throughout the body. Highly expressed in skeletal muscle and brain with weaker expression in heart, kidney and liver, ENT4 functions to transport monoamine molecules, such as dopamine and serotonin, to various areas of the brain. ENT4 is thought to catalyze the reuptake of these molecules into presynaptic neurons, thereby regulating neural signaling events. Although the activity of ENT4 is not dependent upon the intracellular concentrations of ions such as calcium and sodium, its activity is thought to be sensitive to changes in membrane potential. Multiple isoforms of ENT4 are expressed due to alternative splicing events.

REFERENCES

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- Baldwin, S.A., et al. 2004. The equilibrative nucleoside transporter family, SLC29. *Pflugers Arch.* 447: 735-743.
- Engel, K. and Wang, J. 2005. Interaction of organic cations with a newly identified plasma membrane monoamine transporter. *Mol. Pharmacol.* 68: 1397-1407.
- Barnes, K., et al. 2006. Distribution and functional characterization of equilibrative nucleoside transporter-4, a novel cardiac adenosine transporter activated at acidic pH. *Circ. Res.* 99: 510-519.
- Endo, Y., et al. 2007. Cellular localization and functional characterization of the equilibrative nucleoside transporters of antitumor nucleosides. *Cancer Sci.* 98: 1633-1637.
- Bottalico, B., et al. 2007. The organic cation transporters (OCT1, OCT2, Emt) and the plasma membrane monoamine transporter (PMAT) show differential distribution and cyclic expression pattern in human endometrium and early pregnancy decidua. *Mol. Reprod. Dev.* 74: 1303-1311.

CHROMOSOMAL LOCATION

Genetic locus: SLC29A4 (human) mapping to 7p22.1.

PRODUCT

ENT4 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 ENT4 shRNA Plasmid (h): sc-89525-SH and ENT4 shRNA (h) Lentiviral Particles: sc-89525-V as alternate gene silencing products.

For independent verification of ENT4 (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-89525A, sc-89525B and sc-89525C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

ENT4 siRNA (h) is recommended for the inhibition of ENT4 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

ENT4 (SA-18): sc-101295 is recommended as a control antibody for monitoring of ENT4 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 ENT4 gene expression knockdown using RT-PCR Primer: ENT4 (h)-PR: sc-89525-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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