



# VACHT siRNA (h): sc-36803

## BACKGROUND

Neurotransmission depends on the regulated exocytotic release of chemical transmitter molecules. This requires the packaging of these substances into the specialized secretory vesicles of neurons and neuroendocrine cells, a process mediated by specific vesicular transporters. The family of genes encoding the vesicular transporters of monoamines (VMAT 1 and VMAT 2) and acetylcholine (VACHT) have been cloned and functionally characterized. The sequence of these integral membrane proteins predicts twelve transmembrane domains and weak homology to a class of bacterial antibiotic resistance proteins. The vesicular transport of neurotransmitter molecules has been shown to be an active ATP- and proton dependent transport mechanism.

## REFERENCES

1. Roghani, A., et al. 1994. Molecular cloning of a putative vesicular transporter for acetylcholine. *Proc. Natl. Acad. Sci. USA* 91: 10620-10624.
2. Henry, J.P., et al. 1994. Biochemistry and molecular biology of the vesicular monoamine transporter from chromaffin granules. *J. Exp. Biol.* 196: 251-262.
3. Haigh, J.R., et al. 1994. Acetylcholine active transport by rat brain synaptic vesicles. *Neuroreport* 5: 773-776.
4. Yelin, R., et al. 1995. The pharmacological profile of the vesicular monoamine transporter resembles that of multidrug transporters. *FEBS Lett.* 377: 201-207.
5. Varoqui, H., et al. 1996. Active transport of acetylcholine by the human vesicular acetylcholine transporter. *J. Biol. Chem.* 271: 27229-27232.
6. Varoqui, H., et al. 1997. Vesicular neurotransmitter transporters. Potential sites for the regulation of synaptic function. *Mol. Neurobiol.* 15: 165-191.
7. Reimer, R.J., et al. 1998. Vesicular neurotransmitter transport and the presynaptic regulation of quantal size. *Curr. Opin. Neurobiol.* 8: 405-412.

## CHROMOSOMAL LOCATION

Genetic locus: SLC18A3 (human) mapping to 10q11.23.

## PRODUCT

VACHT 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see VACHT shRNA Plasmid (h): sc-36803-SH and VACHT shRNA (h) Lentiviral Particles: sc-36803-V as alternate gene silencing products.

For independent verification of VACHT (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-36803A, sc-36803B and sc-36803C.

## PROTOCOLS

See our web site at [www.scbt.com](http://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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

VACHT siRNA (h) is recommended for the inhibition of VACHT 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  $\mu$ M in 66  $\mu$ 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

VACHT (H-10): sc-518260 is recommended as a control antibody for monitoring of VACHT 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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) Immunoprecipitation: use Protein A/G PLUS-Agarose: sc-2003 (0.5 ml agarose/2.0 ml). 3) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 VACHT gene expression knockdown using RT-PCR Primer: VACHT (h)-PR: sc-36803-PR (20  $\mu$ l). 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.