

## Product Information

### ANTI- VESICULAR ACETYLCHOLINE TRANSPORTER (VACHT)

Developed in Rabbit  
Affinity Isolated Antibody

Product Number **V5387**

#### Product Description

Anti-Vesicular Acetylcholine Transporter (VACHT) is developed in rabbits using a synthetic peptide (K-SPPGPFDCEDDYNYSRS) corresponding to the C-terminal of the cloned rat VACHT (amino acids 512-530 with N-terminally added lysine) conjugated to KLH as immunogen. This sequence is highly conserved (>85% homology) in human VACHT. Antibody to VACHT is affinity-purified using the immunogenic peptide immobilized on agarose.

Anti-Vesicular Acetylcholine Transporter (VACHT) reacts specifically with VACHT (~70 kDa). In immunoblotting VACHT may appear as a doublet band at 67-70 kDa. Staining of the VACHT band by immunoblotting is specifically inhibited with the immunizing peptide (VACHT rat, amino acids 512-530 with N-terminally added lysine).

Anti-Vesicular Acetylcholine Transporter (VACHT) may be used to detection and localization of VACHT by immunoblotting, immunohistochemistry and immunofluorescence.

Vesicular Acetylcholine Transporter (VACHT), (~70kD protein), belongs to the family of vesicular monoamine transporters (VMATs), which include VMAT1 and VMAT2 and the *C. Elegans* putative ACh transporter *unc-17*.<sup>1</sup> Members of this family function to concentrate neurotransmitters into synaptic vesicles through exchange of protons for neurotransmitters. VACHT is a functional transporter for the neurotransmitter acetylcholine (ACh). ACh is synthesized in the cytoplasm by choline acetyl transferase (ChAT) and transported by VACHT into synaptic vesicles where it is stored until released.<sup>1,7</sup> After release from presynaptic nerve terminals ACh is hydrolyzed by extracellular ACh-esterases (AChE) to choline and acetate.

The genes for human and rat VACHT have been cloned.<sup>2,3</sup> Analysis of the *VACHT* gene has revealed a single genetic locus encoding both *VACHT* and *ChAT*, providing a unique genomic arrangement suggesting that these genes may be co-regulated. VACHT mRNA is expressed in all known major cholinergic neurons in the central and peripheral nervous system.<sup>2,3</sup> The amino acid sequence of VACHT suggests a protein with 12 transmembrane domains, containing a large intravesicular loop between transmembrane domains I and II. VACHT is abundantly expressed in the CNS and is mainly localized in small synaptic vesicles in cholinergic nerve terminals.<sup>4,8</sup> VACHT provides a specific marker for cholinergic neurons<sup>5,6</sup> for the study of cholinergic transmission in experimental models, in Alzheimer's disease and other nervous system disorders.

#### Reagents

The product is provided as a solution in 0.01 M phosphate buffered saline pH 7.4 containing 1% BSA and 15 mM sodium azide as preservative.

#### Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

#### Storage/Stability

For continuous use, store at 2-8 °C for up to one month. For extended storage freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

### Product Profile

A minimum working dilution of 1:2,000 is determined by immunoblotting using a cytosolic fraction of rat brain extract.

A minimum working dilution of 1:1000 is determined by immunohistochemistry of 4% paraformaldehyde perfusion-fixed frozen, free-floating sections of rat brain.

A minimum working dilution of 1:500 is determined by immunofluorescent staining of cultured NGF-differentiated PC12 cells fixed with 3% paraformaldehyde.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

### References

1. Usdin, T.B., et al., Trends Neurosci., **18**, 218 (1995).
2. Erickson, J.D., et al., J. Biol. Chem., **269**, 21929 (1994).
3. Roghani, A., et al., Proc. Natl. Acad. Sci. USA, **91**, 10620 (1994).
4. Weihe, E., et al., Proc. Natl. Acad. Sci. USA, **93**, 3547 (1996).
5. Arvidsson, U., J. Comp. Neurol., **378**, 454 (1997).
6. Gilmor, M.L., et al., J. Neurosci., **16**, 2179 (1996).
7. Varoqui, H. and Erickson, J.D., J. Biol. Chem., **273**, 9094 (1998).
8. Varoqui, H. and Erickson, J.D., J. Biol. Chem., **271**, 27229 (1996).

lpg 5/99

Sigma brand products are sold through Sigma-Aldrich, Inc.

Sigma-Aldrich, Inc. warrants that its products conform to the information contained in this and other Sigma-Aldrich publications. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see reverse side of the invoice or packing slip.