ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Vesicular Acetylcholine Transporter (VAChT/VAT)

□ **Cat.** VAT11-S	**Chicken** Anti-Mouse/Rat VAT antiserum	**SIZE**: 100 ul
□ **Cat.** VAT11-A	**Chicken** Anti-Mouse/Rat VAT IgG (affinity pure)	**SIZE**: 100 ug
□ **Cat.** VAT11-P	Mouse/Rat VAT Control peptide	**SIZE**: 100 ug

Acetylcholine (Ach) is synthesized in the cytoplasm of cholinergic neurons through the action of the enzyme choline acetyltransferase, and is then transported into small synaptic vesicles by the vesicular acetylcholine transporter (**VAChT or VAT**). ACh transport involves the exchange of two luminal protons for each molecule of cytoplasmic transmitter transported into the vesicle. The driving force for transport is derived from a proton electrochemical gradient across the vesicular membrane generated by a vacuolar type H+-ATPase located on the synaptic vesicle. The filled vesicles release their contents through exocytosis, producing an action potential at the nerve terminal. As a consequence of this process VAChT is transported with the synaptic vesicle to the plasma membrane where it fuses with the plasma membrane. Synaptic vesicles and their component proteins, including VAChT, are recycled from the plasma membrane back to the synaptic vesicle by endocytosis via an endosomal compartment. The cytoplasmic tail of VAChT contains the signal(s) which traffic it to the synaptic vesicle. The amino acid sequence of VAChT has revealed that it belongs to a family of transporters which includes the monoamine transporters VMAT1 and VMAT2. This family of transporters can be characterized as containing 12-transmembrane domains, an N-linked glycosylated loop located between TM1-2 and cytoplasmic N/C-terminal domains.

Source of Antigen and Antibodies

Antigen	20aa peptide of Rat/Mouse VAT **Designated (VAT11-P or control peptide) Epitope location~ C-terminal, Cytoplasmic domain**
Ab Host/type	Chicken, polyclonal Unpurified antiserum (cat # VAT11-S) Aff pure IgG (**cat #VAT11-A) purified over antigen-agarose column**
2-ab	**Goat Anti-chicken IgG-HRP** cat # 60320 (AP, biotin, FITC conjugates also available)
-ve control IgG	**Cat # 20010-1, Chicken (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control**

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
□ 100ul □ □ solution □ lyophilized powder
Supplied 0.05% azide, **Reconstitute** powder in 100 ul PBS

Affinity pure IgG
□ 100 ug/100ul □ solution □ lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
Reconstitute powder in PBS at 1mg/ml

Control/blocking peptide
□ 100 ug/100 ul □ solution □ lyophilized powder
Supplied in Buffer: PBS pH 7.5,
Reconstitute powder in PBS at 1 mg/ml.

Storage

Short-term: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

Long-term: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

Stability: 6-12 months at –20oC or below.
Shipping: 4oC for solutions and room temp for powder

Recommended Usage

Western Blotting (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure using Chemiluminescence technique).

ELISA (1:100K; using 50-100 ng control peptide/well).

Histochemistry & Immunofluorescence: Not tested. we recommend the use of affinity purified antibody at 2-20 ug/ml in formaldehyde fixed tissue. An antibody made to this VAT11-P has been used to label cholinergic cell bodies in the septum and nucleus basalis and cholinergic fiber (2).

Specificity & Cross-reactivity

The 20-aa mouse/rat VAT11-P sequence shows 93% homology with the human and 91% with monkey VAT (1). No significant sequence homology is seen with other neurotransmitter transporter. Antibody cross-reactivity in various species has not been studied. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity.

General References: **(1)** Roghani A et al 91994) PNAS 91, 10620-10624; Naciff JM et al (1997) Neuroport. 8, 3467-3473; Erickson JD (1994) JBC 269, 21929-21932; **(2)** JBC (1995) 270; 24654; J Neurosci. 16, 2179-2190.

*This product is for in vitro research use only.

Some New Antibodies from ADI…

Western Blot recycling kit (Use the same blot to probe with multiple antibodies) **recycle blot at room temp in 5-10 min; No mercaptoethanol or heating required**).

VAT11-S-A-P 71208A

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