

**RABBIT ANTI-VAMP-2
PURIFIED, POLYCLONAL ANTIBODY**

CATALOG NUMBER:	AB5856-200UL
LOT NUMBER:	
QUANTITY:	200 µL
CONCENTRATION:	1 mg/mL (after reconstitution)
SPECIFICITY:	Recognizes VAMP-2 (Vesicle-Associated Membrane Protein 2, Synaptobrevin 2). The epitope does not share homology with any other known proteins.
IMMUNOGEN:	GST fusion protein corresponding to a cytoplasmic, N-terminal part of rat or mouse VAMP-2 (Accession numbers Q64357, NP 036795).
APPLICATIONS:	Western blot: 1:1,000 using ECL on rat brain membranes. Immunohistochemistry on mouse brain sections. Dilutions should be made using a carrier protein such as BSA (1-3%) Optimal working dilutions must be determined by the end user.
CONTROL ANTIGEN:	Included free of charge with the antibody is 50 µg of control antigen (lyophilized powder). The stock solution of the antigen can be made up using 100 µL of PBS. For positive control, in Western blot using 20 ng of protein per minigel lane. For negative control, preincubate 3 µg of fusion protein with 1 µg of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.
SPECIES REACTIVITIES:	Rat and mouse. It is expected that the antibody will also react with human, <i>Macaca multata</i> and bovine due to sequence homology (25/26). Other species have not been tested.
FORMAT:	Purified immunoglobulin.
PRESENTATION:	Lyophilized from phosphate buffered saline, pH 7.4, containing 1% BSA, 5% sucrose as a stabilizer and 0.025% sodium azide as a preservative. Reconstitute with 200 µL of sterile deionized water. Centrifuge antibody preparation before use (10,000 xg for 5 min).
STORAGE/HANDLING:	Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.
REFERENCE:	Kubista, H., et al., <i>Journal of Cell Science</i> (2003) 117 :955-966.

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SUGGESTED WESTERN BLOT PROTOCOL

1. Mix the samples with sample-buffer X 2, and heat 10 min at 70°C.
2. 5-50 µL applied to Minigel lane (0.75-1.5 mm width) and run at standard conditions. (60 mA for 2 1.5 mm Minigel gels, 1.4 h)
3. Transfer in semi-dry system under standard conditions (3 h 200 mA for two minigel gels)
4. Stain the transferred bands with Chemicon BLOT-*FastStain* (Catalog Number 2076).
5. Destain with deionized water.
6. Block with 5% non-fat milk (Marvel or Carnation) in PBS, and 0.025 % sodium azide, overnight at 4°C. The non-fat milk should be dissolved freshly, centrifuged 10,000 rpm for 10 min, and filtered through glass filter (Gelman Acrodisc).
7. Incubation with first antibody 2 h at room temperature or overnight at 4°C in blocking solution. The antibody preparation should be centrifuged before use (10,000 g 5 min.). Optimal working dilutions and incubation time will need to be determined by the end user.
8. Wash 4 x 15 min. with PBS-0.1% tween 20. From this stage, azide should be omitted.
9. Incubation with the secondary antibody (HRP-conjugated goat anti-rabbit antibody, for example Chemicon Catalog Number AP132P, diluted appropriately) 1 h at room temperature.
10. Wash 4 x 15 min. with PBS-0.1% tween 20.
11. Perform ECL with commercial kits (*ChemiLUCENT*, Chemicon Catalog Number 2600).

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