

**RABBIT ANTI-BRAIN TYPE II VOLTAGE GATED SODIUM CHANNEL (Na<sub>v</sub>1.2)  
AFFINITY PURIFIED  
POLYCLONAL ANTIBODY**

<b>CATALOG NUMBER:</b>	AB5206
<b>LOT NUMBER:</b>	
<b>QUANTITY:</b>	200 µL
<b>CONCENTRATION:</b>	0.45 mg/mL (after reconstitution)
<b>SPECIFICITY:</b>	Recognizes type II α subunit of VGSC (Na <sub>v</sub> 1.2, Scn2a, BII). Does not cross react with any other sodium channel antigens tested so far. Homology with Na <sub>v</sub> 1.3 is 12 or 15 amino acids.
<b>IMMUNOGEN:</b>	Purified peptide of α subunit (amino acids 467-485) of rat type II voltage-gated sodium channel (VGSC) (Accession P04775).
<b>APPLICATIONS:</b>	<p><i>All procedures that are going to receive a full-length protein should be performed at 4 °C, and the following protease inhibitor mixture should be used: pepstatin A (1 µg/mL), leupeptin (1 µg/mL), aprotinin (1 µg/mL), Pefabloc SC (0.2 mM), benzamidine (0.1 mg/mL), and calpain inhibitors I and II (8 µg/mL each).</i></p> <p>Western blot: 1:200 using ECL on rat brain membranes. <u>Note:</u> The addition of 2% Triton to the antibody is recommended for this lot number. Immunohistochemistry on rat brain sections. Immunoprecipitation on rat brain lysates (6 µg) Dilutions should be made using a carrier protein such as BSA (1-3%) Optimal working dilutions must be determined by the end user.</p>
<b>CONTROL ANTIGEN:</b>	Included free of charge with the antibody is 40 µg of control antigen (lyophilized powder). The stock solution of the antigen can be made up using 100 µL of sterile deionized water. For negative control, preincubate 1 µg of peptide with 1 µg of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.
<b>SPECIES REACTIVITIES:</b>	Rat. Other species have not been tested. The immunogen sequence is identical with human.
<b>FORMAT:</b>	Affinity purified immunoglobulin.
<b>PRESENTATION:</b>	Lyophilized from phosphate buffered saline, pH 7.4, containing 1% BSA, and 0.05% sodium azide as a preservative. Reconstitute with 200 µL of sterile deionized water. Centrifuge antibody preparation before use (10,000 xg for 5 min).
<b>STORAGE/HANDLING:</b>	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.
<b>REFERENCE:</b>	Iwata, A., et al, <i>Journal of Neuroscience</i> (2004) <b>24</b> :4605-4613.

## SUGGESTED WESTERN BLOT PROTOCOL

1. Mix the samples (organ membranes: 50 µg/lane; transfected cells: 500,000 cells/lane) 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). It is suggested that you run 5-15% acrylamide (37.5:1 acrylamide:bisacrylamide) minigel (1.5 mm width) at 30 mA/gel ~1-1.5 hours.
3. Transfer in semi-dry system under standard conditions (3 h 100 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 2-8°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 10 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 10 min. with PBS-0.1% tween 20.
11. Perform ECL with commercial kits (Chemilucifer, Chemicon Catalog Number 2600).

## PROTOCOL FOR IMMUNOHISTOCHEMISTRY

Sacrifice and tissue processing

Rats are deeply anesthetized with pentobarbital sodium (Pental). Brain is fixed by transcardial perfusion, first with 50 mL of phosphate buffered saline (0.02M PBS, pH 7.4) containing heparin (5U/ml), then with 220 mL of ice-cold 4% paraformaldehyde in 0.1M PBS, pH 7.5 containing sucrose 4%. Brain is cut in coronal blocks and further fixed by immersion in the same fixative, refrigerated, for 1-2 hours. Brain blocks are transferred to 10% sucrose in 0.1M PBS and sectioned in a cryostat within 3 days. Brain sections, 30 µm thick, are floated in 0.1M PBS and then preserved in a cryopreservation buffer at -20°C. The cryopreservation buffer contains 40% ethylene glycol and 1% polyvinylpyrrolidone in 0.1M potassium acetate buffer, pH 6.5.

Staining procedure

1. Floating sections are rinsed in 0.02M PBS, 2x 5 minutes.
2. Endogenous peroxidase activity is quenched by incubation with 0.2 % hydrogen peroxide in 0.1M phosphate buffer pH 7.3 containing 0.2% Triton X-100 for 25 minutes at room temperature.
3. Sections are rinsed in 0.02M PBS, 2x 5 minutes.
4. Sections are incubated with the primary antiserum in a medium containing 0.3% Triton X-100, 0.05% Tween 20, 4% normal donkey serum (NDS), for 1 hour at room temperature and then overnight refrigerated.
5. Sections are rinsed in 0.02M PBS, containing 4% NDS, 2x 5 minutes.  
Sections are incubated with biotinylated donkey anti-rabbit (from Chemicon USA, catalog number AP182B) diluted 1:400 in 0.02M PBS, containing 0.3% Triton X-100, 0.05% Tween 20, and 4% NDS, for 1 hour at room temperature and then overnight refrigerated.
  1. Sections are rinsed in 0.02M PBS containing 4% NDS, 2x 5 min.
  2. Sections are incubated with extravidin-peroxidase (Sigma Catalog number E2886) diluted 1:100 in 0.02M PBS, for 45 minutes at room temperature.
  3. Sections are rinsed in 0.02M PBS, 3x 5 minutes.

Color development

1. Sections are incubated with a solution of diaminobenzidine at the concentration of 0.0125% and containing 0.05% nickel ammonium sulfate for 10 minutes at room temperature.
2. Sections are transferred to the same DAB solution but with added hydrogen peroxide at a final concentration of 0.0015%. Duration of incubation should be adjusted by the end user.
3. Sections are rinsed in 0.02M PBS, 4x 10 minutes.
4. Sections are mounted on glass slides (gelatinized or coated by any other type of adhesive material) and allowed to dry.
5. Sections are dehydrated in ascending series of ethanol concentrations (70%, 90%, 100%, 5 minutes in each), delipidated in xylene (10 minutes) and coverslipped in Permount (or any other xylene diluted adhesive).

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