

**RABBIT ANTI-BRAIN TYPE I VOLTAGE GATED SODIUM CHANNEL
AFFINITY PURIFIED
POLYCLONAL ANTIBODY**

- CATALOG NUMBER:** AB5204
- LOT NUMBER:** 22100913
- QUANTITY:** 200 μ L
- CONCENTRATION:** 0.3 mg/mL (after reconstitution)
- SPECIFICITY:** Recognizes type I α subunit of VGSC. Does not cross react with any other sodium channel antigens tested so far.
- IMMUNOGEN:** Purified peptide from 465-481 of α subunit of rat type I voltage-gated sodium channel (VGSC) (Accession P04774).
- APPLICATIONS:** *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 μ g/mL), leupeptin (1 μ g/mL), aprotinin (1 μ g/mL), Pefabloc SC (0.2 mM), benzamidine (0.1 mg/mL), and calpain inhibitors I and II (8 μ g/mL each).*
Western blot: 1:200 using ECL on rat brain membranes.
Immunohistochemistry on rat 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 40 μ g of control antigen (lyophilized powder). The stock solution of the antigen can be made up using 100 μ L of sterile deionized water. For negative control, preincubate 1 μ g of peptide with 1 μ g 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 may also react with human due to sequence homology. Other species have not been tested.
- FORMAT:** Affinity 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 μ L 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 reconstitution maintain at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

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 (*ChemiLUCENT*, Chemicon Catalog Number 2600).

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