

**RABBIT ANTI- Ca_v2.3 (α 1E VOLTAGE GATED CALCIUM CHANNEL, R-Type)
AFFINITY PURIFIED
POLYCLONAL ANTIBODY**

CATALOG NUMBER:	AB5248-50UL
LOT NUMBER:	
QUANTITY:	50 μL
CONCENTRATION:	0.3 mg/mL (after reconstitution)
SPECIFICITY:	Recognizes all forms of Ca _v 2.3 (α 1E, R-type of voltage-gated calcium channel, Cacna1e). Does not cross react with any other calcium channel antigens tested so far.
IMMUNOGEN:	Purified peptide from amino acids 892-907 of rat Ca _v 2.3.
APPLICATIONS:	<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> 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 20 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.
SPECIES REACTIVITIES:	Rat. Other species have not been tested. The immunogen sequence is 14/16 identical in mouse, 12/16 in rabbit and 11/16 in human.
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 50 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.

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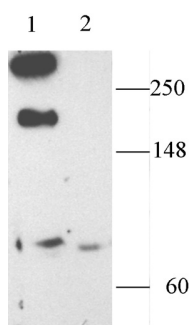
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SUGGESTED WESTERN BLOT PROTOCOL

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 (Chemiluminescent, Chemicon Catalog Number 2600).



Western blotting of rat brain membranes:

1. Anti- $Ca_v2.3$ (1:200)
2. Anti $Ca_v2.3$, preincubated with the control peptide

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