

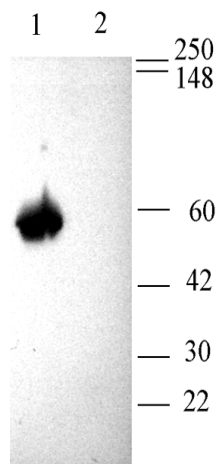
**RABBIT ANTI-CALCIUM CHANNEL BETA 3
AFFINITY PURIFIED
POLYCLONAL ANTIBODY**

CATALOG NUMBER:	AB5230-200UL
LOT NUMBER:	
QUANTITY:	200 μ L
CONCENTRATION:	0.3 mg/mL (after reconstitution)
SPECIFICITY:	Recognizes the full length Ca β ₃ protein, not with other known proteins tested so far.
IMMUNOGEN:	A purified peptide corresponding to amino acid residues 463-477 of rabbit Ca β ₃ (Accession number P54286).
APPLICATIONS:	Western blot: 1:200-1:300 using ECL on rat brain membranes. 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 purified 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. 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 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.

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SUGGESTED WESTERN BLOT PROTOCOL

1. Mix the samples (organ membranes: 50 μ g/lane; transfected cells: 500,000 cells/lane) with sample-buffer X 2, and heat 10 min at 70°C.
2. 5-50 μ L 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 (*Chemil*LUCENT, Chemicon Catalog Number 2600).



Western blotting of rat brain membranes (25 μ g/lane)

1. AB5230 (1:200)
2. AB5230 preincubated with control peptide

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