

**RABBIT ANTI-GABA(A)  $\alpha$ 1**  
**( $\gamma$ -aminobutyric acid receptor type A  $\alpha$ 1 subunit, GABRA1)**  
**AFFINITY PURIFIED, POLYCLONAL ANTIBODY**

<b>CATALOG NUMBER:</b>	AB5592-200UL
<b>LOT NUMBER:</b>	
<b>QUANTITY:</b>	200 $\mu$ L
<b>CONCENTRATION:</b>	0.8 mg/mL (after reconstitution)
<b>SPECIFICITY:</b>	Recognizes GABA(A) $\alpha$ 1. The epitope shares little homology with $\alpha$ 5 and $\alpha$ 2 subunits (respectively, 9/16 and 8/16 residues identical). Does not share homology with any other known proteins.
<b>IMMUNOGEN:</b>	Peptide corresponding to amino acids 28-43 from rat GABA(A) $\alpha$ 1 subunit (Accession P18504).
<b>APPLICATIONS:</b>	Western blot: 1:200 using ECL on rat brain membranes. Immunohistochemistry on rat brain tissue sections. Dilutions should be made using a carrier protein such as BSA (1-3%) Optimal working dilutions must be determined by the end user.
<b>CONTROL ANTIGEN:</b>	Included free of charge with the antibody is 40 $\mu$ g of control antigen (lyophilized powder). The stock solution of the antigen can be made up using 100 $\mu$ L of sterile deionized water. For negative control, preincubate 1 $\mu$ g of peptide with 1 $\mu$ g of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.
<b>SPECIES REACTIVITIES:</b>	Rat. It is expected that the antibody will also work on mouse. The epitope is highly conserved in human and bovine (16/17) and chicken (14/17). Other species have not been tested.
<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 $\mu$ L 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.

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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 2-8°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).

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