

RABBIT ANTI-GABA(A) α 3
(γ -aminobutyric acid receptor type A α 3 subunit, GABRA3)
AFFINITY PURIFIED, POLYCLONAL ANTIBODY

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|------------------------------|---|
| CATALOG NUMBER: | AB5594-200UL |
| LOT NUMBER: | |
| QUANTITY: | 200 μ L |
| CONCENTRATION: | 0.3 mg/mL (after reconstitution) |
| SPECIFICITY: | Recognizes GABA(A) α 3. The epitope does not share homology with any other known proteins. |
| IMMUNOGEN: | Peptide corresponding to amino acids 1-15 from human GABA(A) α 3 subunit (Accession P34903) (residues 29-43 of the precursor). |
| APPLICATIONS: | 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 μ g of control antigen (lyophilized). 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. It is expected that the antibody will also work on human. The epitope is identical in mouse and highly conserved in bovine (14/15). 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. |

For research use only; not for use as a diagnostic.

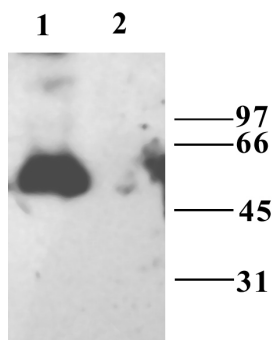
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SUGGESTED WESTERN BLOT PROTOCOL

1. Mix the samples 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)
3. Transfer in semi-dry system under standard conditions (3 h 200 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 4°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 15 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 15 min. with PBS-0.1% tween 20.
11. Perform ECL with commercial kits (*Chem*LUCENT, Chemicon Catalog Number 2600).



Western blotting of rat brain membranes (50 µg)

1. AB5594, 1:200
2. AB5594, preincubated with the control peptide antigen.

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