

**RABBIT ANTI-AGMATINE**  
(Guanidinium Analogue)  
**POLYCLONAL ANTIBODY**

- CATALOG NUMBER:** AB1568-2000T
- LOT NUMBER:** xxxx
- QUANTITY:** 2000 T (500 µL)
- SPECIFICITY:** Agmatine. No detectable cross reactivity with arginine, glutamate or other amino acids.
- IMMUNOGEN:** Agmatine conjugated to BSA
- APPLICATIONS:** Immunohistochemistry: 1:100 on 0.1-2.5% glutaraldehyde fixed tissue. Optimal fixation: 0.1-2.5% glutaraldehyde, 1% formaldehyde. Minimum glutaraldehyde: 0.1%. Works on paraffin embedded tissue (fixed with glutaraldehyde) but preferably epoxy embedded - specifically developed for post-embedding protocols.  
Immunocytochemistry on cells with glutamate-gated ion channels.  
Immunoblotting
- AB1568 has been used successfully on retina tissue fixed in 2.5% glutaraldehyde buffer using 250 nm sections. Assay conditions: Living, isolated goldfish retinas were maintained for 10 minutes in an oxygenated physiological solution that preserves normal neuronal activity under constant lighting. The medium also contained 10 mM agmatine. After the incubation period retinas were fixed in a conventional 2.5% glutaraldehyde buffer and processed for immunohisto-chemistry using 250 nm sections probed with a gold labeled secondary antibody. The treated retinas were exposed to 125 M kainate during the incubation. Kainate opens both AMPA and kainate selective ionotropic channels through which agmatine can enter cells.
- Optimal working dilutions must be determined by the end user.
- FORMAT:** Rabbit antisera.
- PRESENTATION:** Liquid in 100 mM phosphate buffer with 1% goat serum and 0.05% thimerosal.
- STORAGE/HANDLING:** Store at 2-8°C in undiluted aliquots for up to 6 months after date of receipt.
- REFERENCES:** Hille, B. Ionic channels of excitable membranes. (1984) Sinauer Assoc., Sunderland, MA.  
Li, et al., *Science* (1994) **263**  
Quik, M., *Brain Research* (1985) **325**:79-88.  
Yoshikami D., *Science* (1985) **212**:929-930.  
Marc, R.E. et al., *J. Neuroscience* (1995) **15**:5106-5129.  
Marc, R.E. *Society for Neuroscience Abstracts* (1995) 21:pg 1992 #781.6.  
Steullet, P. et al., *J. Comparative Neurology* (2000) **418**:270-280.

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## Rabbit anti-Agmatine (AB1568)

### General processing of Retinas or Brain Slices with AGB loading

#### Protocol

1. Isolate living retinas or cut 400  $\mu$ m brain slices on chopper.
2. Incubate 15 minutes in oxygenated physiological medium (species specific, of course) with 25 mM NaCl replaced by AGB plus any desired pharmacological reagent to modulate AGB permeation.
3. Fix in 3% glutaraldehyde in standard EM grade buffer.
4. Embed in epoxy resin and cut sections to desired thickness. Remember that post-embedding immunocytochemistry is a surface binding event and is thickness independent. Use 40 nm sections for optimal serial amino acid sampling, 90 nm for combined EM/LM sampling, 250 nm sections for routine amino acid sampling and 500 nm for easiest quick surveys.
5. Use AB1568-2000T properly diluted.
6. Process with silver intensification.

### Suggested Immunohistochemical Staining Protocol

#### Stock Solutions

Na Ethoxide

Anhydrous Ethanol (EtOH)

Anhydrous Methanol (MeOH)

Phosphate Buffer (0.1 M, pH 7.4) (PB)

PB + 0.05% thimerosal, pH 7.4 (PBT)

NaIO<sub>4</sub>

Primary Antibody (AB1568-2000T)

Goat anti-Rabbit-gold 1 nm (GAR-gold)

1% Goat serum in PBT (GSPBT)

Silver intensification Stock A - 0.2 M citrate buffer pH 4.85 (critical pH - check carefully)

Silver intensification Stock B - 0.5 g hydroquinone in 15 mL distilled water (dH<sub>2</sub>O)

Silver intensification Stock C - 1% aqueous silver nitrate

Silver intensification Working solution - 5 mL Stock A + 1 mL Stock B + 1 mL Stock C, in order

5% acetic acid

#### Protocol

1. 100-1000 nm sections of epoxy resin embedded tissue.
2. Deplasticize in 1:5 v/v solution of mature saturated ethanolic NaOH (Na ethoxide) in anhydrous EtOH, 1.5 minutes/100 nm section thickness. NO WATER!
3. Wash in three 2 minute changes in anhydrous EtOH or MeOH, one 5 minute running tap water rinse and a dH<sub>2</sub>O dip.
4. Store in PBS until used or dip in dH<sub>2</sub>O, air dry and store.
5. Osmicated specimens (e.g. 1% OsO<sub>4</sub> for 45- 60 minutes) must be treated with fresh 1% NaIO<sub>4</sub> for 7 minutes followed by a 1 minute wash in PBS. Dip in dH<sub>2</sub>O and air dry. Otherwise skip to step 6.
6. Dip in dH<sub>2</sub>O and air dry.
7. Stain with primary antibody diluted in 1% GSPBT (4 hours to overnight), 25  $\mu$ L/well. Sandwich between layers of plastic wrap to prevent evaporation.
8. Flick off primary antibody. Dip once in 0.1 M PBS to rinse off excess primary antibody. Wash one hour in 1% GSPBT; use plastic cassettes, requires about 15 mL of solution.
9. Dip slides in dH<sub>2</sub>O. Rapidly air dry with air canister. Do not get propellant on the slides. Stain with GAR-gold (secondary antibody) properly diluted in 1% GSPBT for 1 hour. Use about 25  $\mu$ L/well.
10. Flick off secondary antibody. Dip in 0.1 M PB to rinse off excess second antibody. Wash in PB 1 hour in plastic cassettes.
11. Dip in dH<sub>2</sub>O and air dry.

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12. Prepare fresh silver intensification solutions.
13. Use the working solution immediately as it only lasts 10 minutes. Use 25 µL of solution/well. Work quickly. Expose sections to the silver intensification solution for 5-7 minutes in a dark vessel (e.g. a tray covered with aluminum foil). If the stain is not very strong by 7 minutes, use two serial 5-7 minute intensifications on the next samples. You must use rigid time protocols for quantitative comparisons.
14. Stop with a brief dip in 5% acetic acid.
15. Wash for 10 minutes in dH<sub>2</sub>O and dry in a dust-free place
16. Cover slip in epoxy resin.

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