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MOUSE ANTI-GLUTAMATE (L-GLUTAMATE) MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB5304

LOT NUMBER:

QUANTITY: 100 µL

SPECIFICITY: Glutamate (L-Glutamate)

The cross-reactivities were determined using an ELISA test by competition experiments with the following compounds:

Compound	Cross-reactivity
L-Glutamate-G-BSA	1
D-Glutamate-G-BSA	1/100
L-Aspartate-G-BSA	1/50,000
GABA-G-BSA	1/50,000

Abbreviations:

(G)	Glutaraldehyde
(BSA)	Bovine Serum Albumin

IMMUNOGEN: Glutamate-Glutaraldehyde-BSA.

ISOTYPE: IgM

APPLICATIONS: Immunohistochemistry: 1:500-1:2,500 using free floating sections by the PAP technique on rat hippocampus.
Optimal working dilutions must be determined by end user.

SPECIES REACTIVITIES: Rat

FORMAT: Purified immunoglobulin.

PRESENTATION: Liquid in PBS containing 10mM sodium azide.

STORAGE/HANDLING: Maintain at -20°C in undiluted aliquots for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles.

REFERENCE: Chagnaud, J.L., et al., *Brain Research* (1989) **481**:175-181.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

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PROTOCOL for Glutamate Detection by Immunohisto/cytochemistry. Example for a rat brain.

1. **SOLUTIONS TO BE PREPARED** - Solution must be prepared as needed.

   Solution A: 0.1M cacodylate, 10g/L sodium metabisulfite, pH 6.2.

   Solution B: 0.1M cacodylate, 10g/L sodium metabisulfite, 3-5% glutaraldehyde, pH 7.5.

2. **RAT PERFUSION** - The rat is anaesthetized with sodium pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with Solution A (30 mL): 150-300 mL/min, Solution B (500 mL): 150-300 mL/min.

3. **POST FIXATION:** 15 to 30 minutes in Solution B, then 4 soft washes in 0.05M Tris with 8.5 g/L sodium metabisulfite, pH 7.5 (Solution C) .

4. **TISSUE SECTIONING:** Vibratome or cryostat sections can be used.

5. **REDUCTION STEP:** Sections are reduced with Solution C containing 0.1M sodium borohydride for 10 minutes. The sections are washed 4 times in solution C without sodium borohydride.

6. **APPLICATION OF GLUTAMATE ANTIBODY:** Use a final dilution of 1:500-1:2,500 in Solution C containing 0.1% Triton X100 and 2% non-specific serum. Incubate 12 sections per 2 mL diluted antibody overnight, +2-8°C. Then wash the sections three times for 10 minutes each in Solution C. (Note that the antibody may be usable at a higher dilution. This should be explored to minimize the possibility of high background. Additionally, note that a change in the buffering system as indicated in the protocol may change the background and antibody recognition). The specific reaction is then revealed by PAP procedure.

6. **SECOND ANTIBODY:** Incubate the sections with a 1:50 to 1:200 dilution of goat anti-mouse in Solution B containing 1% non-specific serum for either 3 hrs at 20°C or 1-2 hr at 37°C. Then wash the sections, 3 times, for 10 minutes each with Solution C.

7. **PAP:** Incubate the sections with the appropriate dilution of peroxidase anti-peroxidase (for free floating method) in Solution C for 1-2 hours at 37°C. Then wash sections 3 times for 10 min each in solution C.

8. **VISUALIZATION:** The antigen-antibody complexes are visualized using DAB-4-HCl (25 mg/100 mL) (or other chromogen) in 0.05M Tris and filtrated; 0.05% hydrogen peroxide is added. Incubate the sections for 10 minutes at room temp. Stop the reaction by transferring the sections to 5 mL 0.05M Tris. Mount sections on chrome-alum coated slides. Dry overnight at 37°C. Rehydrate sections using conventional histological procedures. Coverslip using rapid mounting media.

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FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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