OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Monoclonal Antibody to L-Glutamate - Purified

Catalog No.: AM32616SU-N

Quantity: 0.1 ml

Host / Isotype: Mouse / IgM

Immunogen: Glutamate-Glutaraldehyde-BSA.

Format:
State: Liquid purified IgM fraction
Buffer System: PBS
Preservatives: 10mM Sodium Azide

Applications:
Immunohistochemistry: 1/2,500-1/10,000 using free floating sections by the PAP technique on Rat hippocampus.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity:
Recognizes Glutamate (L-Glutamate).
The cross-reactivities were determined using an ELISA test by competition experiments with the following compounds:
Compound (Cross-reactivity): Glutamate-G-BSA (1), Aspartate-G-BSA (1/100,000), GABA-G-BSA (1/100,000), Glutamate (1/100,000).
G= Glutaraldehyde, BSA= Bovine Serum Albumin
Note: Antibody reactivity REQUIRES glutaraldehyde fixation thus some glutaraldehyde (0.5%-2.0%) needs to be included in the tissue fixation procedure inorder for the proper reactivity.

Species Reactivity: **Tested:** Rat.

Storage:
Store undiluted at 2-8°C.
Shelf life: one year from despatch.

General Readings:
1. Chagnaud, J.L., et al., Brain Research (1989) 481:175-181.

Protocols:

For Glutamate Detection by Immunohisto/cytochemistry. Example for a Rat brain.

1. **Soslutions 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 1:2,500-1:10,000 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.

7. **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.

8. **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.

9. **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.