

# Mouse Anti-Conjugated L-Glutamate Monoclonal Antibody

Catalog No: MA2018

Quantity: 100 μl

**Description:** Monoclonal antibody was obtained after BALB/c mouse immunization with the

conjugates: L-Glutamate-Glutaraldehyde-carrier proteins and hybridization of spleen cells with the myeloma cell line SP2/O/Ag14. Ascite production was performed in

BALB/c mice.

Product Form: Lyophilized

**Reconstitution:** Reconstitute with 50 μl of distilled water and 50 μl of glycerol.

**Purification:** The ascitic fluid was purified by ammonium sulfate precipitation.

Target: Conjugated L-glutamate

**Immunogen:** Synthetic L-glutamate conjugated to protein carrier (Pc)

Isotype: IgM Kappa

**Specificity:** Using a conjugate L-glutamate-(Pc), antibody specificity was performed with an

ELISA test by competition experiments with the following compounds:

Compound Cross-reactivity ratio\*

L-Glutamate-G-(Pc) 1

D-Glutamate-G-(Pc) 1/100 L-Aspartate-G-(Pc) 1/50,000 GABA-G-(Pc) 1/50,000

\* L-Glutamate-G-(Pc) concentration/other conjugated amino acid concentration at half

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displacement:

G = Glutaraldehyde, GABA = Gamma-Aminobutyric Acid

**Applications:** ELISA: working dilution of 1:1,000 – 1:5,000

Immunocytochemistry: working dilution of 1:1,000 – 1:5,000

Western Blot: working dilution of 1:1,000 – 1:2,000

**Related Products:** Cell Sciences sells the corresponding antigen:

AG001: L-glutamate -G -BSA Antigen

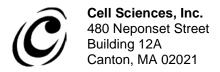
Storage: Lyophilized antibody is stable at least 2 years. Avoid repeated freeze-thaw cycles.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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#### Examples of ELISA protocol used to test conjugated L-Glutamate:

- 1. Coating of conjugated L-glutamate (15 μg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05M (pH 9.6), for sixteen hours at 4°C.
- 2. Saturation of well plates with of a solution of PBS (pH 7.3) containing 2.5 g/l of BSA and 0.05%Tween 20 for one hour at 37°C.
- 3. Wash with PBS Tween (two times).
- 4. Anti-conjugated L-glutamate antibody will be diluted (1:1,000-1:5,000) in PBS containing 2.5 g/l BSA and 10% glycerol, 200 μl by well plate (incubating for 2 hours at 37°C).
- 5. Wash with PBS Tween (three times).
- 6. 200 µl of peroxidase-labelled goat anti-mouse IgG diluted (1:10,000) in a solution of PBS containing 2.5 g/l BSA, 10% glycerol and 0.5% of Tween, will be applied by well plate (for one hour at 37°C).
- 7. Well plates will be rinsed with a PBS Tween (three times).
- 8. Peroxidase will be developed by incubating 200µl by well plate of a citrate 0.1 M phosphate 0.2M (pH 5) solution containing 0.4% OPD (Sigma) and 0.03% hydrogen peroxide for ten minutes in the dark, after that, we will stop the reaction by the addition of 50 µl of 2M HCl.
- 9. The optical density is measured at 492nm.

### Example of Immunocytochemistry application used to test conjugated L-glutamate:

#### **Detection of conjugated Glutamate in rat brain:**

1. **Perfusion**: The rat is anesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions:

solution A (30 ml): 150-300 ml/min solution B (500 ml): 150-300 ml/min

Solution A: cacodylate 0.1M, sodium metabisulfite 10 g/l, pH = 6.2

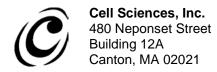
Solution B: cacodylate 0.1M, sodium metabisulfite 10 g/l and glutaraldehyde 3-5%, pH = 7

- 2. **Post fixation**: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05M with sodium metabisulfite 8.5g/l, pH 7.5 (solution C).
- 3. **Tissue sectioning**: Cryostat or vibratome sections can be used.
- 4. **Reduction step**: Sections are reduced with the solution C containing sodium borohydride (0.1M) for 10 min. Then, the sections are washed 4 times with solution C without sodium borohydride.
- 5. **Application of anti-conjugated Glutamate antibody**: The final dilution is 1:1,000 to 1:5,000 in solution C containing 0,1% triton X100, plus 2% of non-specific serum. A dozen sections can be incubated with 2 ml of monoclonal antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution C.
  - <u>Note</u>: The antibody may be used at a higher dilution. The customer should try further antibody dilutions to reduce the possibility of high background. Note that a substitution in the buffer system as used in our protocol may change the background and the antibody recognition.
- 6. PAP procedure:

<u>Second antibody</u>: Sections are incubated with 1:200 dilution of goat anti-mouse in solution C for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;

<u>PAP</u>: Sections are incubated with 1:1,000 dilution of mouse peroxidase/anti-peroxidase complex in solution C for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution C;

 $\frac{\text{Revelation}}{\text{chromogen}} : \text{Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of <math>H_2O_2$  is added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5 ml of 0.05M Tris HCl, ph6.5.



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#### Example of cytochemical applications used to test conjugated Glutamate:

## **Detection of conjugated Glutamate in cockroach brain:**

- 1. **Fixation**: Cockroach brains were fixed overnight at 4°C in fixative comprising 1% glutaraldehyde, 2.5% paraformaldehyde, and 1% sodium metabisulfite (SMB, Sigma) in 0.1M cacodylate buffer adjusted to pH 7.2.
- 2. **After fixation**, whole brains were immersed in 10<sup>-2</sup>M sodium borohydride (NaBH<sub>4</sub>, Sigma) in a solution of 0.05M Tris-HCl buffer with 0.5% SMB pH 7.5.
- **3. Tissue sectioning :** After a wash in 0.05M Tris-HCI-SMB buffer, brains were embedded in 8% agarose for serial 80 µm frontal and sagittal sections.
- 4. **Application of anti-conjugated antiserum**: Sections were incubated with 10% normal swine serum in 0.05M Tris-HCI-SMB with 0.5% TritonX100 (Tx).
  - **Application of anti-conjugated rabbit glutamate antiserum**: Sections were incubated overnight at room temperature in rabbit glutamate antiserum diluted to 1/1000-1/5000.
- 5. Revelation:
  - <u>Second antibody</u>: After a wash in Tris-HCl-Tx, sections were incubated overnight with goat anti-rabbit immunoglobulin conjugated to Texas Red (1/250 Tris-HCl-Tx, Jackson Laboratories). After a final wash in Tris-HCl, the sections were embedded in the 80% glycerol.

To **double label** glutamate and taurine, agarose sections were incubated overnight with mouse monoclonal anti-glutamate antibodies at a dilution of 1/100 together with rabbit polyclonal anti-taurine antibodies (1/500) in Tris-HCI-Tx. After washing, the secondary antibodies goat anti-rabbit immunoglobulin conjugated to Texas Red (1/250) and Alexa 488 goat anti-mouse immunoglobulin conjugate (1/250) were applied simultaneously to the sections for incubation at room temperature overnight. After a 6-8 hours wash, sections were mounted in glycerol.

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