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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 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.
   Note: 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** :
   Second antibody : 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 ;
   PAP : 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 ;
   Revelation : Antibody-antigen complexes are revealed using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.05% of 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^{-2}M sodium borohydride ($NaBH_4$, 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-HCl-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-HCl-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:**
   Second antibody : 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-HCl-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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