Anti-Conjugated L-Glutamate Antibodies

Product Information Sheet
 # 1014GE

SUMMARY

shipped on blue ice; store at -20 °C

For research use only.

Not for use in diagnostic or therapeutic procedures.

Target: Conjugate L-glutamate

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

Raised in: Rabbit

Clonality: Polyclonal

Isotype: IgG

Purity: Antiserum previously preabsobed on protein carriers, and purified.

Form: Lyophilized

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 [(a)]	
L-Glutamate-G-(Pc)	1	
D-Glutamate-G-(Pc)	1:	> 50,000
L-Aspartate-G-(Pc)	1:	> 50,000
D-Aspartate-G-(Pc)	1:	> 50,000
GABA-G-(Pc)	1:	> 50,000

(a): Glutamate-G-(Pc) concentration / other conjugated amino acid concentration at half displacement; G = Glutaraldehyde

Storage Instructions

Lyophilized antibodies are stable at least 2 years. After reconstitution with 50 µl of distilled water and 50 µl of glycerol, the aliquot can be repeated freezed (up to 5 times)

Tested Applications: Elisa, Immunocytochemistry, Immunohistochemistry

Applications Notes

Recommended dilutions for

Elisa	(1:1,000 - 1:5,000)
Immunocytochemistry	(1:1,000 - 1:5,000)
Western Blot	(1:1,000 - 1:2,000)

Research Areas: Neurobiology, Neurodegenerative diseases, Pharmacology, Biochemistry

Examples of Materials and Methods

1. Example of ELISA protocol used to test conjugated L-glutamate:

1. Coating of conjugated L-glutamate (10 µg/ml) in maxisorp well plates (Nunc) with a solution of sodium carbonate buffer 0.05 M (pH 9,6), during sixteen hours at 4 °C.
2. Saturation of well plates with of a solution of PBS (pH 7,3) containing 1 g/l of BSA (Acros), 10% of glycerol and 0.5% of Tween (one hour at 37 °C).
3. Wash with PBS containing 0.5% of Tween (PBS Tween) (3 times).
4. Anti-conjugated L-glutamate antibodies will be diluted (1:1,000 - 1:5,000) in PBS Tween containing 1 g/l BSA, 1g/l of BSA-G and 10% of glycerol, 200 µl by well plate (incubating during 2 hrs at 37 °C).
5. Wash with PBS Tween (3 times).
6. 200 µl of peroxidase-labelled goat anti-rabbit (Jackson) diluted (1:10,000) in a solution of PBS Tween containing 1 g/l of BSA, will be applied by well plate (during one hour at 37 °C) .
7. Well plates will be rinsed with PBS Tween (3 times).
8. And finally the peroxidase will be developed by incubating 200 µl by well plate of a citrate 0,1 M/phosphate 0,2 M (pH 5) solution containing 0.4% of OPD (Sigma) and 0.03% of hydrogen peroxide (Acros) for ten minutes in the dark. After that, stop the reaction by the addition of 50 µl of 2M HCl.
9. The optical density will be mesured at 492 nm.

2. Example of Immunocytochemistry applications used to test conjugated L-glutamate

Detection of conjugated Glutamate in rat brain

1. Perfusion: The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following solutions :
   solution A (30 ml): 200-300 ml/min
   solution B (500 ml): 200-300 ml/min
   Solution A : cacodylate 0.1 M, sodium metabisulfite 10 g/l, pH = 6.2
   Solution B : cacodylate 0.1 M, sodium metabisulfite 10 g/l, glutaraldehyde 3-5%, pH = 7.5

2. Post fixation: 15 to 30 min in solution B, then 4 soft washes in Tris 0.05 M with sodium metabisulfite 8.5 g/l, pH 7.5 (solution C).

3. Tissue sectionning: Cryostat or vibratome sections can be used.

4. Application of anti-conjugated Glutamate antibodies: The final dilution is 1:1,000 to 1:5,000 in solution C containing Triton X100 0.5%, plus 2% of non-specific serum. A dozen of sections can be incubated with 2 ml of antibody solution overnight at 4 °C. Then, after this period, the sections are washed 3 times (10 min) with solution C.
   N.B.: Antibodies may be used at a higher dilution. The customer should explore the antibody dilution 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.

5. PAP procedure
   Second antibody: Sections are incubated with 1/100 dilution of goat anti-rabbit in solution C for 3 hrs at 20 °C or 1 hr at 37 °C. Then, they are washed 3 times (10 min) with solution C; PAP: Sections are incubated with 1:1,000 dilution of rabbit peroxidase anti-peroxidase complex in solution C for 1 hr at 37 °C. Then, they are washed 3 times (10 min) with solution C;
   Revelation: Antibody-antigen complexes are revealed using diaminobenzidine (25 mg/100 ml) (or other chromogen) dissolved in Tris 0.05 M and filtrated ; 0.05% of H_2O_2 is added. The sections are incubated for 10 min at 20 °C. Reaction is stopped by transfering sections in 5 ml of Tris 0.05 M.

References

1. RAJAOFETRA N., PASSAGIA J.G., MARLIER L., POULAT P., PELLAS F., SANDILLON F., VERSCHUERE B., GOUY D., GEFFARD M. and PRIVAT A. Serotoninergic, noradrenergic and peptidergic innervation of onuf's nucleus of normal and transected spinal cords of Baboos (PapioPapio). J. of Comp. Neurol., (1992), 318, 1-17.
2. SINAKEVITCH-PEAN I., PLOTNIKOVA S.I., GEFFARD M., BOCKAERT J. and GRAU Y. Glutamate-like immunoreactivity in the adult brain of *Drosophila melanogaster*. European Journal of Neurosciences (1998) 10, 285.
3. SINAKEVITCH-PEAN I., GEFFARD M. and PLOTNIKOVA S.I. Glutamate-like immunoreactivity in the central nervous system of *Drosophila melanogaster*. Journal of Evolutionnary Biochemistry and Physiology (2001) 37(1), 64-68.
4. SINAKEVITCH I., M.FARRIS S.and STRAUSFELD J. Taurine-, aspartate-, glutamate-like immunoreactivity identifies chemically distinct subdivisions of kenyon cells in the cockroach mushroom body.

Order Information, Shipping and Storage

Order#	Product	Quantity
1014GE	Anti-Conjugated L-Glutamate Antibodies	50 µl
1031GE	Anti-Conjugated Glutamate, monoclonal	50 µl
shipped on blue ice; store at -20 °C		

Contact and Support

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