

Mouse Anti-Conjugated Acetylcholine Monoclonal Antibody

Catalog No: MA2026

Size: 100 µl / vial

Description:

Monoclonal antibody to acetylcholine was obtained after AKR mouse immunization with the conjugates : Choline-Glutaric anhydride-carrier proteins and hybridization of spleen cells with the myeloma cell line SP2/O/Ag14. Ascite production was performed in irradiated BALB/c mice.

Purification:

The ascitic fluid was purified by ammonium sulfate precipitation and gel filtration.

Storage and handling:

Monoclonal antibody is lyophilized with 0.01% merthiolate and is stable at +4°C. Reconstituted antibody with 25µl or 50µl of distilled water (written on the bottle) can be stored at +4°C.

Specificity:

Using a conjugate Choline-Glutaric anhydride-Protein, antibody specificity was performed with an ELISA test by competition experiments with the following compounds :

Compound	Cross-reactivity ratio (a)
Choline-GA-BSA	1
Choline-AA-BSA	1/536
Choline-SA-BSA	1/2,500
Farnesol-BSA	1/> 100,000
Retinoic acid-BSA	1/> 100,000
Myristic acid-BSA	1/> 100,000
Acetylcholine	1/> 100,000
Choline	1/> 100,000

(a) Choline-GA-BSA concentration/unconjugated or conjugated compounds concentration at half displacement
GA Glutaric anhydride
AA Adipic acid
SA Succinic acid
BSA Bovine Serum Albumin

Subclass : IgG 2a, Kappa

Recommended dilution:

The antibody was tested using the free floating PAP technique on rat cholinergic areas. The anti-conjugated acetylcholine antibody gave a good staining between a 1/500-1/5,000 dilution in these areas.

Applications:

Immunohistochemistry, immunocytochemistry.

References

- CHAGNAUD J.L., SOUAN M.L., CHARRIER M.C. and GEFFARD M. Monoclonal anti-conjugated acetylcholine antibody and immunohistochemical applications in rat nervous. J. Neurochem., (1989) 53, 383-391.



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Immunohisto and cytochemical applications

Detection of conjugated Acetylcholine in rat brain

Perfusion : The rat is anaesthetized with sodium Pentobarbital or Nembutal and perfused intracardially through the aorta using a pump with the following fixative solution : glutaraldehyde 0.5M, 2-nitrobenzyl alcohol 0.1M, sodium metabisulfite 10g/l and cacodylate 0.1M, sodium bromide 0.01M, pH 10.7 (solution A : 200-300ml/min).

Post fixation : 2h in 0.5M glutaraldehyde solution (pH 7.5) without the 2-nitrobenzyl alcohol, then 4 soft washes in Tris 0.05M with sodium metabisulfite 10g/l, pH 7.4 (solution B).

Tissue sectioning : Cryostat or vibratome sections can be used.

The sections were washed 4 times in solution B, and incubated for 1h at 37°C in solution B containing 0.2% triton X100, plus 1% of non specific serum.

Application of anti-conjugated Acetylcholine antibody : The final dilution is 1/500 to 1/5,000 in solution B containing 0,2% triton X100, plus 1% of non-specific serum. A dozen sections can be incubated with 2ml of antibody solution overnight at 4°C. Then, after this period, the sections are washed 3 times (10 min) with solution B.

N.B. : The antibody may be used at a higher dilution. The customer should explore the further 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.

PAP procedure :

Second antibody : Sections are incubated with 1/200 dilution of goat anti-mouse antibodies diluted at 1/100 in solution B for 3 hours at 20°C or 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution B.

PAP : Sections are incubated with mouse peroxidase/anti-peroxidase complex diluted at 1/500 in solution B for 1 hour at 37°C. Then, they are washed 3 times (10 min) with solution B.

Detection : Antibody-antigen complexes are detected using diaminobenzidine (25mg/100ml) (or other chromogen) dissolved in Tris 0.05M and filtrated ; 0.3% of nickel ammonium sulfate ; 0.05% of H₂O₂ were added. The sections are incubated for 10 min at 20°C. Reaction is stopped by transferring sections in 5ml of Tris 0.05M.

References

- CHAGNAUD J.L., SOUAN M.L., CHARRIER M.C. and GEFFARD M. Monoclonal anti-conjugated acetylcholine antibody and immunohistochemical applications in rat nervous system. J. Neurochem., (1989) 53, 383-391.

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