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RABBIT ANTI-M2 MUSCARINIC ACETYLCHOLINE RECEPTOR AFFINITY PURIFIED POLYCLONAL ANTIBODY

CATALOG NUMBER: AB5166-200UL

LOT NUMBER:

QUANTITY: 200 µL

CONCENTRATION: 0.85 mg/mL (after reconstitution)

SPECIFICITY: Recognizes a full-length m2 protein. It has exhibited no cross reactivity with other muscarinic proteins tested so far.

IMMUNOGEN: GST fusion protein and part of i3 intercellular loop of human m2 muscarinic acetylcholine receptor (amino acids 225-356) (Accession P08172).

APPLICATIONS: Western blot: 1:200 using ECL on rat brain membranes.
Immunohistochemistry on rat brain sections.
Immunoprecipitation
Dilutions should be made using a carrier protein such as BSA (1-3%)
Optimal working dilutions must be determined by the end user.

CONTROL ANTIGEN: Included free of charge with the antibody is XX µg of control antigen. The stock solution of the antigen can be made up using 100 µL of PBS. For positive control, in Western blot using 10 ng of protein per minigel lane. For negative control, preincubate 3 µg of fusion protein with 1 µg of antibody for one hour at room temperature. Optimal concentrations must be determined by the end user.

SPECIES REACTIVITIES: Human, mouse and rat. Other species have not been tested. In Chimpanzee and gorilla the immunogen sequence is 100% identical. Orangutan (130/132), pig (122/132), and dog (122/132).

FORMAT: Affinity purified immunoglobulin.

PRESENTATION: Lyophilized from phosphate buffered saline, pH 7.4, containing 1% BSA, and 0.05% sodium azide as a preservative. Reconstitute with 200 µL of sterile deionized water. Centrifuge antibody preparation before use (10,000 xg for 5 min).

STORAGE/HANDLING: Maintain lyophilized material at -20°C for up to 12 months after date of receipt. After reconstitution maintain at -20°C in undiluted aliquots for up to 6 months. Avoid repeated freeze/thaw cycles.

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SUGGESTED WESTERN BLOT PROTOCOL

1. Mix the samples (organ membranes: 50 μg/lane; transfected cells: 500,000 cells/lane) with sample-buffer X 2, and heat 10 min at 70°C.
2. 5-50 μL applied to Minigel lane (0.75-1.5 mm width) and run at standard conditions. (60 mA for 2 1.5 mm Minigel gels, 1.4 h). It is suggested that you run 5-15% acrylamide (37.5:1 acrylamide:bisacrysmide) minigel (1.5 mm width) at 30 mA/gel ~1-1.5 hours.
3. Transfer in semi-dry system under standard conditions (3 h 100 mA for two minigel gels)
4. Stain the transferred bands with Chemicon BLOT-*FastStain* (Catalog Number 2076).
5. Destain with deionized water.
6. Block with 5% non-fat milk (Marvel or Carnation) in PBS, and 0.025 % sodium azide, overnight at 2-8°C. The non-fat milk should be dissolved freshly, centrifuged 10,000 rpm for
   10 min, and filtered through glass filter (Gelman Acrodisc).
7. Incubation with first antibody 2 h at room temperature or overnight at 4°C in blocking solution. The antibody preparation should be centrifuged before use (10,000 g 5 min.). Optimal working dilutions and incubation time will need to be determined by the end user.
8. Wash 4 x 10 min. with PBS-0.1% tween 20. From this stage, azide should be omitted.
9. Incubation with the secondary antibody (HRP-conjugated goat anti-rabbit antibody, for example Chemicon Catalog Number AP132P, diluted appropriately) 1 h at room temperature.
10. Wash 4 x 10 min. with PBS-0.1% tween 20.
11. Perform ECL with commercial kits (Chemilucent, Chemicon Catalog Number 2600).

PROTOCOL FOR IMMUNOHISTOCHEMISTRY

Sacrifice and tissue processing

Rats are deeply anesthetized with pentobarbital sodium (Pental). Brain is fixed by transcardial perfusion, first with 50 mL of phosphate buffered saline (0.02M PBS, pH 7.4) containing heparin (5U/ml), then with 220 mL of ice-cold 4% paraformaldehyde in 0.1M PBS, pH 7.5 containing sucrose 4%. Brain is cut in coronal blocks and further fixed by immersion in the same fixative, refrigerated, for 1-2 hours. Brain blocks are transferred to 10% sucrose in 0.1M PBS and sectioned in a cryostat within 3 days. Brain sections, 30 µm thick, are floated in 0.1M PBS and then preserved in a cryopreservation buffer at -20°C. The cryopreservation buffer contains 40% ethylene glycol and 1% polyvinylpyrrolidone in 0.1M potassium acetate buffer, pH 6.5.

Staining procedure

1. Floating sections are rinsed in 0.02M PBS, 2x 5 minutes.
2. Endogenous peroxidase activity is quenched by incubation with 0.2 % hydrogen peroxide in 0.1M phosphate buffer pH 7.3 containing 0.2% Triton X-100 for 25 minutes at room temperature.
3. Sections are rinsed in 0.02M PBS, 2x 5 minutes.
4. Sections are incubated with the primary antiserum in a medium containing 0.3% Triton X-100, 0.05% Tween 20, 4% normal donkey serum (NDS), for 1 hour at room temperature and then overnight refrigerated.
5. Sections are rinsed in 0.02M PBS, containing 4% NDS, 2x 5 minutes.

Sections are incubated with horseradish peroxidase labeled donkey anti-rabbit (from Chemicon USA, catalog number AP182P), 1:400 in 0.02M PBS, containing 0.3% Triton X-100, 0.05% Tween 20, and 4% NDS for 1 h at room temperature and then refrigerated overnight.

1. Sections are rinsed in 0.02M PBS containing 4% NDS, 2x 5 min.
2. Sections are rinsed in 0.02M PBS, 2x 5 minutes.

Color development

1. Sections are incubated with a solution of diaminobenzidine at the concentration of 0.0125% and containing 0.05% nickel ammonium sulfate for 10 minutes at room temperature.
2. Sections are transferred to the same DAB solution but with added hydrogen peroxide at a final concentration of 0.0015%. Duration of incubation should be adjusted by the end user.
3. Sections are rinsed in 0.02M PBS, 4x 10 minutes.
4. Sections are mounted on glass slides (gelatinized or coated by any other type of adhesive material) and allowed to dry.
5. Sections are dehydrated in ascending series of ethanol concentrations (70%, 90%, 100%, 5 minutes in each), delipidated in xylene (10 minutes) and coverslipped in Permount (or any other xylene diluted adhesive).

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

FOR RESEARCH USE ONLY; NOT FOR USE IN DIAGNOSTIC PROCEDURES. NOT FOR HUMAN OR ANIMAL CONSUMPTION

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