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MOUSE ANTI-CHOLINE ACETYLTRANSFERASE MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB5270-100UG (formerly Roche 1372432)

LOT NUMBER:

QUANTITY: 100 μg

CONCENTRATION: 1 mg/mL

SPECIFICITY: The antibody reacts with choline acetyltransferase (ChAT)-positive cells from humans, rats and pigs [2, 6].

IMMUNOGEN: Highly purified ChAT obtained from porcine brain [1].

ISOTYPE: IgG_1

CLONE NAME: 1.B3.9B3

APPLICATIONS: Western blot
ELISA
Immunohistochemistry: 10-20 μg/mL
Optimal working dilutions must be determined by end user.

SPECIES REACTIVITIES: Human, rat, and porcine.

FORMAT: Purified immunoglobulin.

PRESENTATION: Liquid. Buffer = 0.02 M Phosphate buffer, 0.25 M NaCl with 0.1% sodium azide.

STORAGE/HANDLING: Maintain antibody at 2-8°C in undiluted aliquots for up to 6 months.

REFERENCES:

1. Ostermann et al. (1990) *Eur. J. Biochem.* **192**, 215.
2. Ostermann-Fatif et al. (1992) *Journal of Biochemistry.* **58**, 1060.
3. Watson et al. (1986) *Peptides* **7***,* 155.
4. Eckenstein, F. & Thoenen, H. (1982) *EMBO J.* **1**, 363.
5. Houser, C. R. et al. (1983) *Brain Res.* **266**, 97.
6. Ostermann-Fatif et al. (1992) *Journal of Immunological Methods.* **157**, 73.

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Immunohistochemistry Protocol for MAB5270

Solutions:

I. Buffer A. 100 mM sodium phosphate, pH 7.2
   Buffer B. 100 mM Tris-HCI, 150 mM NaCI, pH 7.4.
II. Heparin solution: 0.05 Heparin (w/v) in buffer A.
III. Paraformaldehyde-picric acid solution: 4% Paraformaldehyde (w/v), 15% saturated picric acid solutions (v/v) in buffer A.
IV. Buffer for anti-choline acetyltransferase: 5% fetal calf serum (FCS) (w/v), 0.1–0.5% Triton X-100 (v/v) in Buffer B.
V. Anti-choline acetyltransferase: Dilute reconstituted antibody solution 1:5 (human) or 1:10 (rat) with solution IV (= 10–20 μg antibody/ml).

VI. Wash Buffer - Buffer B
VII. Anti-mouse-Ig in buffer IV
VIII. PAP solution in buffer IV
IX. Substrate solution: Diaminobenzidine, 1 mg/ml, 0.01% H_2O_2 in buffer A.

Stability of solutions

Solution I is stable for 1 week at 2-8°C.
Solutions II and III should be prepared immediately prior to use.
Solutions IV–VII are stable for 1 week at 2-8°C.
Solution VIII should be prepared daily.

Test principle

During the initial incubation step, the anti-choline acetyltransferase antibody binds to the enzyme. Subsequent to removal of excess antibody, anti-mouse Ig is applied as brigde antibody. The preparation is then incubated in peroxidase-anti-peroxidase solution. The reaction can be visualized diaminobenzidine.

Fixation

a) Human tissue: Fix by placing fresh (5–8 h post mortem) tissue in a 1:1 solution of 4% paraformaldehyde/10% formaldehyde for 1 week at 2-8°C. Divide into tissue blocks (basal forebrain, septum and basal ganglia). Incubate the blocks in sucrose solutions of increasing concentration (10%, 20%, and 30%). Prepare 50 μm sections and store in a cryoprotective solution at -15°C (3).
b) Rat tissue: Brain and spinal cord marrow tissue (transcardial perfusion, modification of the method as described by Eckenstein & Thoenen (4) and Houser et al. (5). Perform a transcardial perfusion initially with oxygen-saturated heparin solution (II) (100–200 ml) and subsequently with paraformaldehyde/picric acid solution (III) (400 ml). Carry out immediate tissue preparation and fix for a further 30 minutes in the same solution. Wash out the fixative with several fresh quantities of buffer A. Prepare 50 μm sections.

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Preparation of sections

1. Block the endogenous peroxidase with 3% H_2O_2 (v/v), and 10% methanol (v/v) and incubate in buffer B for 20 min at 15-25°C.
2. Wash sections three times with buffer B.
3. Incubate sections in solution IV for 30 min at 15-25°C.
4. Wash sections three times with buffer B.
5. Incubate sections in antibody solution V (human tissue: overnight at 2-8°C) (rat tissue: 2 h at 37°C)
6. Wash sections three times with buffer B.
7. Incubate sections in solution VI for 60 min at 15-25°C.
8. Incubate sections in solution VII for 60 min at 15-25°C.
9. Wash sections three times with buffer B.
10. Incubate sections in substrate solution IX at 15-25°C until a red-brown color is clearly visible. A negative control section incubated in parallel should remain colorless.
11. Wash the sections in buffer B.
12. Mount the sections on chromalaun coated slides, allow to dry, dehydrate and embed.

The sensitivity and speed of the test can be influenced by varying the temperature, incubation time and amount of reagent used. Hence, individual requirements can be taken into account. The sodium azide used is a strong peroxidase inhibitor and should thus be used in the primary anti-ChAT antibody solution only. 0.05% thimerosal (w/v) can be used instead.

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