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MOUSE ANTI-CHOLINE ACETYLTRANSFERASE (ChAT) MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB305

LOT NUMBER:

QUANTITY: 100 µL

SPECIFICITY: Cholinergic neurons in the brain and central nervous system.

IMMUNOGEN: Choline acetyltransferase purified from rat brain.

ISOTYPE: IgG_1

CLONE NAME: 1E6

APPLICATIONS: Immunohistochemistry: 1:100-1:250. See page 2.
Optimal working dilutions must be determined by the end user.

SPECIES REACTIVITY: Rat. It has also been reported that the antibody will recognize chick (13 & 14) and human (18).

FORMAT: Ascites fluid.

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

REFERENCES:

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2. *PNAS USA* (1982) **79**:7031-7035.
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4. *Brain Res.* (1987) **402**:30-43.
5. *Exp. Neurology* (1988) **101**:303-312.
6. *Brain Research* (1989) **505**:29-38.
7. *Exp. Neurology* (1990) **109**:153-163.
8. *J. Electron Microscopy Technique* (1990) **15**:2-19.
9. *Brain Research* (1991) **552**:320-329.
10. *Neuron* (1992) **8**:145-158.
11. *J. of Neuroscience* (1998) **18**(9):3351-3362.
12. *J. of Neuroscience* (1998) **18**(9):3124-3137.
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14. *J. Comparative Neurology* (1999) **410**:457-466.
15. *Neuroscience* (1999) **89**:759-770.
16. *Cell Tissue Research* (1999) **297**:409-421.
17. Rymar, V. et al., *J. Comparative Neurology* (2004) **469**:325-339.
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IMMUNOHISTOCHEMISTRY PROCEDURE (PAP TECHNIQUE) FOR MAB305, MONOCLONAL ANTIBODY TO CHOLINE ACETYLTRANSFERASE

I) Perfusion & Sectioning Procedure

1. Perfuse through the heart with a fixative solution containing 4% paraformaldehyde in 0.12 M phosphate buffer (pH 7.3) for light microscopy (LM), and additionally, 0.1% gluteraldehyde and .002% $CaCl_2$ for electron microscopy (EM).
2. Remove brain and postfix 2-18 hours at 4°C in 4% paraformaldehyde in 0.12 M phosphate buffer.
3. After brain is blocked for sectioning, wash in several changes of buffer for 2-3 hours.
4. Specimens for EM are sectioned on a Vibratome (50 µm) and rinsed in buffer, those for LM should be cryoprotected in 30% sucrose in buffer.
5. After freezing with dry ice, 30-40 µm thick sections of LM specimens are cut on a cryostat.
6. Sections are rinsed, and then stored in phosphate buffer containing 0.1% sodium azide.

II) Staining Procedure

Tissue is processed as freely-floating sections in continuously agitated solutions. All incubations are performed at room temperature unless otherwise stated.

1. a. <u>For localizing ChAT-positive somata and dendrites:</u>
   Sections are washed in 0.1 M Tris-buffered saline (TBS; containing 1.4% NaCl, pH 7.3) only. No detergent or enzyme pretreatment is used.
   b. <u>For localizing ChAT-positive terminal-like structures:</u>
   Incubate sections in TBS (pH 8.1) for 5 minutes at 37°C. Transfer sections to TBS (pH 8.1) containing pronase (1.2 µg/mL) for 1 1/2-2 minutes at 37°C, followed by several ice cold buffer washes for a total of 5 minutes. The concentration of pronase and incubation time of the digestion should be evaluated for each region examined.
   c. <u>For localizing ChAT immunoreactivity and subsequently counterstaining the sections:</u>
   Incubation in TBS containing 0.1%-0.8% Triton X-100 for 15 minutes may increase the tissue penetration of the immunoreagents, but it also raises the background staining.
2. Incubate sections in normal goat serum (3-5%) for one hour. The working solutions of all antisera should also contain similarly diluted normal goat serum.
3. Incubate in anti-ChAT monoclonal antibody solution (Suggested working dilution 1:250, final working dilution must be determined by end user) for 2 hours at room temperature and then for an additional 6-18 hours at 4°C.
4. Incubate with second antibody (i.e. Goat anti-Mouse IgG, Cat. No.: AP124, dilution 1:50-100) for 1-2 hours.
5. Incubate with diluted PAP complex (i.e. Mouse PAP, Cat No.: PAP14, conc. 25-50 µg/mL) for one hour.
6. After rinsing in buffer, the second antibody and PAP steps are repeated for 40 minutes to 1 hour each in order to amplify staining intensity, particularly of small ChAT-containing structures.
7. React for 15 minutes with 0.06% 3,3'-diaminobenzidine×4 HCl (DAB; diluted in phosphate buffered saline, pH 7.3) and 0.006% H_2O_2.
8. Specimens for routine LM are postfixed for 1 minutes in 0.005% OsO_4 (osmium tetraoxide), and then mounted, dehydrated and coverslipped. Selected regions blocked for EM are postfixed in 2% OsO_4 for 1 hour, <u>en bloc</u> stained with uranyl acetate, and flat-embedded in Epon-Araldite resin.

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PROCEDURE REFERENCES:

Houser, C.R., et al., (1983) *Brain Res.* **266**:97-119.
Houser, C.R., et al., (1984) *J. Histochem. Cytochem*. **32**:395-402.
Phelps, P.E., et al., (1985) *J. Comp. Neurol*. **283**:286-307.

Important Note: *During shipment, small volumes of antibody will occasionally become entrapped in the seal of the product vial. For antibodies 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.*

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