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GOAT ANTI-CHOLINE ACETYLTRANSFERASE (ChAT) AFFINITY PURIFIED POLYCLONAL ANTIBODY

CATALOG NUMBER: AB144P-1ML **QUANTITY:** 1 mL

LOT NUMBER:

SPECIFICITY: Choline acetyltransferase found in cholinergic neurons in brain and central nervous systems.

IMMUNOGEN: Human placental enzyme.

APPLICATIONS: Immunohistochemistry at 1:100. See attached protocol.
Immunoblot at 1:100-1,000. Expected M.W. 68-70 kD.
Optimal working dilutions must be determined by end user.

SPECIES REACTIVITY: Rat, mouse, macaque monkey, guinea pig, chicken, opossum, avian and human (immunoblot).

FORMAT: Affinity purified immunoglobulin.

PRESENTATION: Liquid in PBS containing 5 mg/mL BSA and 0.2% sodium azide.

STORAGE/HANDLING: Maintain at -20°C in undiluted aliquots for up to 12 months after date of receipt. Avoid repeated freeze/thaw cycles. For short term storage (3-4 weeks) maintain at +2-8°C.

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Immunocytochemistry Procedure (PAP Technique) For Goat Anti-ChAT Affinity Purified Polyclonal Antibody: AB144P

I) **Reagents** - all dilutions, including that of the antibodies, must be determined empirically by the laboratory performing the staining.

1. Goat anti-ChAT (Chemicon AB144P).
2. Secondary anti-sera (anti-Gt IgG, Chemicon AP106).
3. PAP reagent (Gt PAP, Chemicon PAP 10).
4. Diluent for antisera [PBS +0.1% Triton + 3% normal serum (same host as second antibody), i.e. rabbit serum (Chemicon S20)].
5. Buffers: PBS or Tris buffered saline, pH 7.4-7.6.
6. 0.05% DAB (3,3'-diaminobenzidine tetrahydrochloride). Dissolve 25 mg DAB in 50 mL Tris buffer, filter under vacuum using Nalgene filters, and add 0.2 mL of 3% H_2O_2. Use within 30 min. You may intensify the reaction product by adding 1g nickel ammonium sulfate to this solution before filtering. The free base form of DAB may also be used. Caution: neutralize all DAB solution and glassware with bleach, and wear gloves when weighing.

II) **Perfusion procedure**

1. Flush with 100-150 mL saline (physiologic pH) at room temperature.
2. Fix with 300-500 mL of fixative over 30 min. The use of 4% paraformaldehyde + 0.1-0.2% gluteraldehyde is suggested. Other fixatives that have been used successfully include 4% paraformaldehyde in 0.1M phosphate buffer (pH 7.4) or 4% paraformaldehyde + 15% saturated picric acid + 0.08% gluteraldehyde in 0.1M phosphate buffer (pH 7.4).
3. Incubate brain overnight at +4°C in 1:1 fixative (without gluteraldehyde): buffer. (If necessary, the brain may be sectioned after 1 hour in the solution). Transfer to 25% sucrose in 0.1M phosphate buffer pH 7.4 until brain sinks (18-24 hours).

III) **Sectioning**

Vibratome sections, 30-40 microns (or if brain is cryoprotected, a freezing microtome can be used). Collect in 0.1M phosphate buffer (pH 7.4). [24 well culture plates make good collection and storage vessels]. If sections are to be stored, transfer to cryoprotectant [150g sucrose + 300 mL ethylene glycol + volume of 0.05M phosphate buffer (pH 7.4) to make 1 liter] and store at -20°C.

IV) **Staining Procedure Notes**

A. All incubation steps should be carried out using free-floating sections at +4°C, except steps 2, 12-16 which are done at room temperature.
B. Perform all steps on a shaker table.
C. PBS in rinse steps should be at +4°C; keep in refrigerator in squeeze bottle.

Staining Procedure

1. Rinse 3x3 minutes each with PBS.
2. Incubate with 0.4% Triton in PBS at room temperature for 30 minutes.

Staining Procedure (CONT)

3. Rinse 3x3 minutes each with PBS.
4. Incubate with diluent (for reduced background add 1-5% BSA to diluent) for 60 minutes.
5. Rinse 3x3 minutes each with PBS.
6. Incubate with primary antisera diluted in diluent 12-24 hours (longer incubations may give better results).
7. Rinse 5x5 minutes each with PBS.
8. Incubate with second antiserum diluted in diluent for 1-2 hours.
9. Rinse 5x5 minutes each with PBS.
10. Incubate with PAP diluted in diluent for 1-2 hours*.

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Staining Procedure (cont)

11. Rinse 5x5 minutes each with PBS.
12. Rinse 3x5 minutes each with TRIS.
13. Incubate in 0.05% DAB solution for 10 minutes.
14. React in 0.05% DAB solution + 0.01% H_2O_2 (34 μL of 30% H_2O_2/100mL DAB solution) in TRIS for 6-8 minutes.
15. Stop reaction with 5x1mL minute each rinse in PB.

Steps 16-19 are optional. The osmication step makes the DAB reaction product visible under darkfield optics, and the post-fixation step eliminates fracturing of the sections when drying on slides after osmication.

16. Post-fix in 1% gluteraldehyde in PB for 15 minutes.
17. Rinse 5x5 minutes in PB.
18. Osmicate in 1% OxO4 in PB for 30-60 seconds.
19. Rinse 5x5 minutes in PB.
20. Mount out of PB onto gelatinized slides and air dry.
21. Dehydrate in a series of EtOH (50-70-95-95-100-100%) for 5 minutes each and clear in two changes of Histosol or xylene for 60 minutes each, then coverslip in DPX.

*--For a double bridge procedure, steps 8-10 are repeated, using 50% higher dilutions of reagents as were used in the first bridging step, and 1 hour incubations.

As an alternative to the unlabeled antibody (PAP) technique, the Avidin-Biotin Conjugate procedure can be used, although the PAP technique appears to be more sensitive. If the ABC 'kit' is used, omit diluent from all solutions subsequent to the primary antiserum incubation and follow the manufacturer's recommendations.

(Footnote: Good results are also seen with Zamboni's Fixative)

Miilipore offers a comprehensive line of secondary antibodies, including fluorescein, rhodamine, peroxidase, alkaline phosphatase, Cy3 and Cy5 conjugates. We also offer species-absorbed conjugates for dual labeling to minimize nonspecific background staining. Ask your Millipore Customer Service Representative for more information about Millipore's secondary antibody conjugates today!

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