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GOAT ANTI-CHOLINE ACETYLTRANSFERASE (ChAT) POLYCLONAL ANTIBODY

CATALOG NUMBER: AB144

LOT NUMBER:

QUANTITY: 50 µL

SPECIFICITY: Cholinergic neurons in brain and central nervous systems.

IMMUNOGEN: Human placental enzyme.

APPLICATIONS: Immunoblot: 1:1,000-1:5,000
Immunohistochemistry: 1:500-1:1,000. See page two of this data sheet.
Optimal working dilutions must be determined by the end user.

SPECIES REACTIVITY: Mouse, guinea pig and rat.

FORMAT: Goat antisera, liquid, no preservatives.

STORAGE/HANDLING: Maintain at -20ºC in undiluted aliquots for up to 12 months. Avoid repeated freeze/thaw cycles.

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Immunocytochemistry Procedure (PAP Technique) For Anti-ChAT Polyclonal Antisera: AB143 (Rb x ChAT) AB144 (Gt x ChAT)

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

1. Rabbit or Goat anti-ChAT. (Millipore AB143 or AB144).
2. Secondary anti-sera (anti-Rb IgG, Millipore AP132, or anti-Gt IgG, Millipore AP106).
3. PAP reagent (Rb peroxidase-anti-peroxidase, Millipore PAP18 or Gt PAP, Millipore PAP10).
4. Diluent for antisera (PBS + 0.1% Triton + 3% normal serum (same host as second antibody), ie. goat serum (Millipore S26) or rabbit serum (Millipore 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_20_2. Use within 30 minutes. You may intensify the reaction product by adding 1 g 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 minutes. 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 +4ºC 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 (Free floating sections)**

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 -20ºC.

IV) **Staining Procedure Notes**

A. All incubation steps should be carried out using free-floating sections at +4ºC, 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 +4ºC, 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.
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*.
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_20_2 (34 µl of 30% H_20_2/100 mL DAB solution) in TRIS for 6-8 minutes.
15. Stop reaction with 5x1 mL 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% OsO4 in PB for 30-60 sec.
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 manufactures recommendations.

(Foot-note: Good results are also seen with Zamboni's Fixative)

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.*

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

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