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**Antibody to Choline Acetyltransferase (ChAT)
RABBIT POLYCLONAL**

Catalog Number: AB-N34

Immunostaining Protocol:

1. Rats were deeply anesthetized with pentobarbital 100 mg/kg and transcardiac perfused with 300 ml of PBS containing 1,000 units/liter heparin sodium (Wyeth Labs), 1.5 g sodium nitrite/liter (Sigma Chemical) then 1 liter of 4% paraformaldehyde in 100mM sodium phosphate pH 7.6.
2. Spinal cord tissue was equilibrated overnight in 30% sucrose prior to sectioning at 40 μ m on a freezing sliding microtome and collected in .05 M TBS.
3. Block tissue in 5% donkey serum (diluted in 0.1% tris-saline triton) for .5 hr.
4. Spinal cord sections were then incubated in anti-Chat #88-5 diluted 1:4000 (in .1% tris-saline triton) for 4 hours at RT with gentle agitation.
5. Wash 3X in .05 M TBS.
6. Incubate sections with diluted secondary biotinylated antibody for 15 min at RT with gentle agitation. Note: dilute secondary antibody and ABC reagent in 5% donkey serum to reduce background staining.
7. Wash as in step 5.
8. Incubate sections for 15 minutes with Vectastain® Elite ABC reagent at RT with gentle agitation.
9. Wash as in step 5.
10. Incubate sections in peroxidase substrate solution (nickel-enhanced diaminobenzidine was used as chromagen) for 5 min. at RT with gentle agitation.