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# NB120-17413 Protocol

## Immunohistochemistry Protocol for GABA Antibody (NB120-17413)

Immunohistochemistry:

Brain tissues were fixed with 4% paraformaldehyde and 0-0.5% glutaraldehyde give good results.

1. Tissues are fixed with 0.1M phosphate buffer, pH 6.5, 4% paraformaldehyde, 0-0.5% glutaraldehyde, 0.5% potassium dichromate. Tissue post-fixed overnight.
2. Cut sectioned in 50um.
3. Incubated in 0.05M Tris buffer, pH 6.5 for 3 hrs.
4. Sections are incubated for 18-24 hours with NB120-17413 diluted 1:500 in PBS, 0.1% sodium azide, 0.2% Triton X-100, 1% normal goat serum.
5. Fluorescein conjugated antibody or PAP may be used as the secondary reagent.

Note: Without colchicine pretreatment well-stained cell bodies are visible in the cerebral cortex, cerebellar cortex, superior colliculus and some brainstem raphe. With colchicine pretreatment, additional cell body staining is present in the interpeduncular nucleus and the dorsal column nuclei. Staining was blocked by preabsorbing with 100uM GABA conjugated to glutaraldehyde.