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Product datasheet for BM106

Choline Acetyltransferase (CHAT) Mouse Monoclonal Antibody [Clone ID: 1E6]

Product data:

Product Type:	Primary Antibodies
Clone Name:	1E6
Applications:	IHC
Recommended Dilution:	Immunohistochemistry on frozen tissues: recommended dilution 1:100 -1:250. (Staining procedure - see "Protocols").
Reactivity:	Human, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	ChAT purified from rat brain
Specificity:	This antibody is specific for choline acetyltransferase and stains cholinergic neurons in the brain and central nervous system.
Formulation:	State: Ascites State: Liquid ascitic fluid
Conjugation:	Unconjugated
Storage:	Aliquot and store at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	choline O-acetyltransferase
Database Link:	Entrez Gene 290567 RatEntrez Gene 1103 Human P28329
Background:	ChAT is an enzyme, present in the presynaptic ends of axons, that catalyzes the transfer of the acetyl group of acetyl CoA to choline,forming the neurotransmitter acetylcholine.
Synonyms:	Choline O-acetyltransferase, Choline acetylase, CHOACTase, ChAT, EC=2.3.1.6

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Note:

Protocol: I Perfusion and Sectioning Procedure

1. Perfuse through the heart with a fixative solution containing 4% paraformaldehyde in 0.12M phosphate buffer (pH 7.3) for light microscopy (LM) and additionally, 0.1% gluteraldehyde and 0.002% CaCl2 for electron microscopy (EM).
2. Remove brain and postfix 2-18 hours at 4? C in 4% paraformaldehyde in 0.12M 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 (50µm) and rinsed in buffer; those for LM should be cryoprotected in 30% sucrose in buffer.
5. After freezing with dry ice, 30-40µm 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) For localising ChAT-positive somata and dendrites:
Sections are washed in 0.1M Tris-buffered saline (TBS, containing 1.4% NaCl, pH 7.3) only. No detergent or enzyme pre-treatment is used.

1. (b) For localising ChAT-positive terminal-like structures:
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 1½-2 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.

1. (c) For localising ChAT immunoreactivity and subsequently counterstaining the sections:
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; optimal dilution should be determined by the 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, dilution 1/50 - 1/100) for 1-2 hours.
5. Incubated with diluted PAP complex (i.e. mouse PAP 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 one 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 PBS pH 7.3) and 0.006% H2O2.
8. Specimens for routine LM are post-fixed for 1 minute in 0.005% OsO4 (osmium tetroxide) and then mounted, dehydrated and coverslipped. Selected regions blocked for EM are postfixed in OsO4 for 1 hour, en bloc stained with uranyl acetate and flat-embedded in Epon-Araldite resin.

Protein Families: Druggable Genome

Protein Pathways: Glycerophospholipid metabolism