

MOUSE ANTI-CNPase
2', 3'-cyclic nucleotide 3'-phosphodiesterase
MONOCLONAL ANTIBODY

- CATALOG NUMBER:** MAB326R (formerly Roche Catalog Number 1442007)
- LOT NUMBER:**
- QUANTITY:** 100 µg
- CONCENTRATION:** 1 mg/mL
- SPECIFICITY:** Anti-CNP reacts with both CNP1 and CNP2 in many species.
- BACKGROUND:** The enzyme 2', 3'-cyclic nucleotide 3'-phosphodi-esterase (CNP) is expressed at high levels by oligodendrocytes in the central nervous system and by Schwann cells in the peripheral nervous system (1). By virtue of this cell-specific expression, CNP is recognized as a characteristic marker for these two myelin-producing glial cell types (1-4). Beyond its enzymatic activity of cleaving the 2', 3'-cyclic terminus of nucleotides (1), the physiological role of CNP is still under investigation. CNP activity has been correlated with myelin and myelin formation, and a dramatic decrease in CNP activity is associated with demyelinating diseases such as multiple sclerosis (1). This enzyme is composed of two proteins, CNP1 (46 kD) and CNP2 (48 kD) (1,5). Although the ratio of CNP1/CNP2 may vary from species to species, their shared primary sequence is conserved phylogenetically. CNP has recently been localized to human chromosome 17 by amplification of somatic cell hybrid DNA using the polymerase chain reaction (PCR) and by Southern blotting of *Hind* III genomic DNA digests (6). Since anti-CNP reacts with a highly conserved region of the enzyme, it can be considered as pan-anti-CNP (5). Anti-CNP can be used as a marker to identify Schwann cells and oligodendrocytes in cell culture and in tissue sections, as well as to localize CNP in cell membrane fractions. As CNP is expressed relatively early in postnatal development, anti-CNP is especially useful for the early identification of oligodendrocytes.
- IMMUNOGEN:** Purified human 2', 3'-cyclic nucleotide 3'-phosphodiesterase
- ISOTYPE:** IgG₁
- CLONE NAME:** 11-5B
- APPLICATIONS:** Western blot: (10 µg/mL antibody, prepared fresh daily)
Immunocytochemistry: (10 µg/mL antibody, prepared fresh daily)
Immunohistochemistry: (10 µg/mL antibody, prepared fresh daily)
Optimal working dilutions must be determined by end user.
- SPECIES REACTIVITIES:** Human, bovine, canine, sheep, mouse, rabbit, and rat (5).
- FORMAT:** Purified immunoglobulin.
- PRESENTATION:** Liquid. Buffer = 0.02M Phosphate buffer, pH 7.6, 0.25M NaCl with 0.1% sodium azide.
- STORAGE/HANDLING:** Maintain at 2-8°C in undiluted aliquots for up to 6 months after date of receipt.

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REFERENCES:

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4. McMorris, F. A., Kim, S. U. and Sprinkle, T. J. (1984) *Brain Res.* **292**:123.
5. Sprinkle, T. J. *et al.* (1987) *Brain Res.* **426**:349.
6. Sprinkle, T. J. *et al.* (1991) *Soc. Neurosci. Abstr.* **17**.1:376.
7. Sprinkle, T. J., Zaruba, M. E. and McKhann, G. M. (1978) *J. Neurochem.* **30**:309.
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9. Falini, B. and Taylor, C. R. (1983) *Arch. Pathol. Lab. Med.* **107**:105.
10. Harlow, E. and Lane, D. (1988) *Antibodies: A Laboratory Manual*, p. 359, Cold Spring Harbor Laboratory, N.Y.
11. Taylor, C. R. (1978) *Arch. Pathol. Lab. Med.* **102**:113.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μ L 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.*

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SUGGESTED PROTOCOLS FOR MAB326R

Immunocytochemistry: The procedure below was developed to localize CNP in primary oligodendrocyte cultures. Perform all steps at room temperature unless otherwise indicated. This procedure represents suggested guidelines for the use of anti-CNP. An antibody concentration of 10 µg/mL works well for most applications. The antibody should be diluted in PBS containing 1% (v/v) normal animal serum and 0.1% (w/v) BSA. Working dilutions of anti-CNP should be freshly prepared on the day of use. Antibody concentrations and incubation conditions for a given experimental system should be determined empirically.

Fix coverslip cultures

1. Fix coverslip in cold 100% (v/v) methanol for 7 min at -20°C.
2. Post-fix coverslip cultures in 4% (v/v) paraformaldehyde for 10 min at room temperature.
3. Wash three times with fresh PBS, 4 min per wash.

React coverslip cultures with Anti-CNP

1. Block non-specific binding by incubating coverslip cultures for 30 min in PBS containing 1% (v/v) normal animal serum and 0.1% (w/v) BSA.
2. Gently dispense 100 µL of appropriately diluted anti-CNP monoclonal antibody onto the surface of the cells.
3. Incubate in a humid chamber for 60 min.
4. Wash three times with PBS, 4 min per wash.

Detection with secondary antibody

1. Gently dispense 100 µL of FITC-conjugated goat anti-mouse IgG F(ab')₂ fragment in a suitable dilution in PBS containing 1% (v/v) normal animal serum and 0.1% (w/v) BSA onto the surface of the cells. Peroxidase-conjugated anti-mouse IgG F(ab')₂ can also be used at 1:200 dilution.
2. Incubate for 60 min.
3. Wash three times with PBS, 4 min per wash.

Other secondary antibody detection systems can be used (8–10). For optimal results, both the primary and secondary antibody reagents should be titrated (11).

Mount and apply coverslip

Place a small drop of aqueous mounting medium on a glass slide. Invert the coverslip, and carefully lower it onto the drop of mounting medium.

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Immunohistochemistry: The procedure below was developed to localize CNP in rat brain tissue sections. Perform all steps at room temperature unless otherwise indicated. Where normal serum is indicated, use normal serum from the same species as the source of the secondary antibody. This procedure represents suggested guidelines for the use of anti-CNP. Antibody concentrations and incubation conditions for a given experimental system should be determined empirically.

Fix and prepare brain sections

1. Perfuse rats (10- to 18-days old) with 4% (v/v) paraformaldehyde, 0.34% (v/v) L-lysine, 0.05% (v/v) sodium m-periodate (4% PLP).
2. Post-fix for 2–4 h in 4% (v/v) PLP.
3. Transfer the brain to PBS containing 30% (w/v) sucrose, and incubate (with gentle agitation) at +4°C for 48–60 h.
4. Using a frozen sliding microtome, cut 25 µm sections of cerebrum and cerebellum. As the sections are cut, collect them in a vial of PBS.

Pretreat sections

1. Incubate sections in methanol containing 0.3% (v/v) hydrogen peroxide for 15 min with gentle shaking.
2. Rinse sections 6 times in PBS, 10 min per rinse.
3. Incubate sections in PBS containing 1 mg/mL sodium borohydride for 20 min with gentle shaking, frequently pipetting the solution onto sections.
4. Rinse sections 8 times in PBS, 10 min per rinse, frequently pipetting the PBS onto sections until no bubbling is observed.

Anti-CNP staining

1. Block non-specific binding by incubating sections in PBS containing 1.5% (v/v) normal serum for 2 h.
2. Incubate sections with anti-CNP (diluted to a final concentration of 10 µg/mL, in PBS containing 1.5 % (v/v) normal serum and 0.3% (v/v) Triton X-100 overnight at +4°C with gentle agitation.
3. Rinse sections 6 times in PBS, 10 min per rinse.
4. Detect with a standard secondary antibody detection system (8–11).
5. Mount sections, dehydrate, and apply coverslips.

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Western blot:

Anti-CNP can be used to detect 2', 3'-cyclic nucleotide 3'-phosphodiesterase on western blots. A standard protocol for anti-CNP is summarized below.

Gel Electrophoresis

1. Dilute antigen (*e.g.*, rat whole brain protein) in sample buffer to 2 mg/ml, and load onto an SDS homogeneous gel (12.5% acrylamide) alongside molecular weight markers (14.3–200 kD).
2. After separation by electrophoresis, transfer the proteins onto a nitrocellulose membrane.

Detection

1. Block the membrane with blocking solution [*e.g.* 2.5% non-fat powdered milk in Tris buffered saline (TBS) containing 0.1% (v/v) Tween-20] for 10 minutes.
2. Incubate the membrane with 10 µg/mL anti-CNP diluted in blocking solution for 1 hour.
3. On a shaker platform, wash the membrane for 10 min in TBS containing 0.1% (v/v) Tween-20.
4. On a shaker platform, wash the membrane for 10 min in blocking solution.
5. Incubate the membrane with alkaline phosphatase-conjugated goat anti-mouse IgG F(ab')₂, appropriately diluted in blocking solution, for 60 min on a shaker platform (For ECL detection methods, secondary antibody solutions will be higher).
6. On a shaker platform, wash the membrane for 10 min in TBS containing 0.1% (v/v) Tween-20.
7. On a shaker platform, wash the membrane for 10 min in TBS.
8. Visualize the complex with a BCIP/NBT color detection system or ECL.

A closely spaced doublet at 46 kD and 48 kD, which may appear as a single band at 46 kD, should be observed.

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