

**#2986** Store at **-20°C**

# CNPase Antibody

100 µl  
 (10 Western mini-blot)

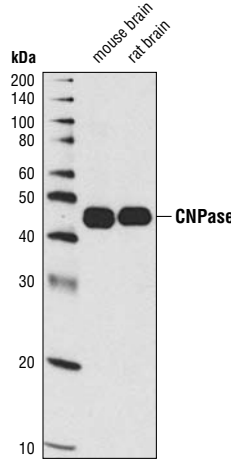
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New 05/08

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Applications	Species Cross-Reactivity*	Molecular Wt.	Source
W Endogenous	H, M, R	47 kDa	Rabbit

**Background:** CNPase (2', 3'-cyclic nucleotide 3'-phosphodiesterase) catalyzes the in vitro hydrolysis of 2', 3'-cyclic nucleotides to produce 2'-nucleotides. The in vivo molecular function and native substrate of this nucleotide phosphodiesterase remains under investigation (1). High CNPase expression is seen in oligodendrocytes and Schwann cells as CNPase accounts for roughly 4% of the total myelin protein in the central nervous system (2). CNPase binds to tubulin heterodimers and plays a role in tubulin polymerization and oligodendrocyte process outgrowth (3). Typical myelination is seen in CNPase knock-out mice, but they suffer severe neurodegeneration from axonal loss and oligodendrocytes display abnormal paranodal loop structure prior to axonal degeneration. Paranodal loops typically contact the axolemma in axon-glia signaling; neurodegeneration in CNPase mice is the secondary consequence of impaired cell-cell communication (4).



*Western blot analysis of extracts from mouse and rat brain using CNPase Antibody.*

**Specificity/Sensitivity:** CNPase Antibody detects endogenous levels of total CNPase protein.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) corresponding to human CNPase. Antibodies are purified by protein A and peptide affinity chromatography.

**Background References:**

- (1) Esposito, C. et al. (2008) *Biochemistry* 47, 308–19.
- (2) Kozlov, G. et al. (2003) *J Biol Chem* 278, 46021–8.
- (3) Lee, J. et al. (2005) *J Cell Biol* 170, 661–73.
- (4) Lappe-Siefke, C. et al. (2003) *Nat Genet* 33, 366–74.

**Entrez-Gene ID** #1267  
**Swiss-Prot Acc.** #P09543

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA and 50% glycerol. Store at **-20°C**.  
*Do not aliquot the antibody.*

**\*Species cross-reactivity is determined by Western blot.**

**Recommended Antibody Dilutions:**

Western blotting 1:1000

**Companion Products:**

- Phospho-DARPP-32 (Thr75) Antibody #2301
- DARPP-32 (19A3) Rabbit mAb #2306
- Doublecortin Antibody #4604
- GFAP (GA5) Mouse mAb #3670
- MAP2 Antibody #4542
- Musashi Antibody #2154
- Neurofilament-L (DA2) Mouse mAb #2835
- Neurofilament-H (RMdO 20) Mouse mAb #2836
- Neurofilament-M (RMO 14.9) Mouse mAb #2838
- Phospho-NMDAR1 (Ser890) Antibody #3381
- PSD95 Antibody #2507
- Phospho-Vimentin (Ser56) Antibody #3877
- Tris Buffered Saline with Tween 20 (TBST - 10X) #9997
- BSA #9998
- Nonfat Dry Milk #9999
- Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
- Anti-rabbit IgG, HRP-linked Antibody #7074
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 20X LumiGLO® Reagent and 20X Peroxide #7003

**IMPORTANT: For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.**

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

### A Solutions and Reagents

**NOTE:** Prepare solutions with Milli-Q or equivalently purified water.

- 1X Phosphate Buffered Saline (PBS)
- 1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
- Nonfat Dry Milk (weight to volume [w/v])
- Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

### B Protein Blotting

A general protocol for sample preparation is described below.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

**NOTE:** CST recommends loading prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

- Incubate membrane with 10 ml LumiGLO<sup>®</sup> (0.5 ml 20X LumiGLO<sup>®</sup>, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

**NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO<sup>®</sup> incubation and declines over the following 2 hours.