Anti-CNPase Antibody [JF10-25]

ET1702-46

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC, IHC-Fr, IF-Tissue
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	JF10-25

Description: 2',3'-cyclic nucleotide-3'-phosphodiesterase (CNPase) is a membrane-bound enzyme that can link tubulin to membranes and may regulate cytoplasmic microtubule distribution. CNPase acts as a microtubule-associated protein by promoting microtubule assembly; this activity resides in the C-terminus of the enzyme. CNPase is firmly associated with tubulin from brain tissue and thyroid cells and can be found at high concentrations in central nervous system myelin and in the outer segments of photoreceptors in the retina. Acute lead intoxication leads to disturbances in CNPase activity and the morphology of myelin.

Immunogen: Synthetic peptide within Human CNPase aa 386-421 / 421.

Positive control: Mouse brain tissue lysate, mouse cerebrum tissue lysate, SHG-44, SH-SY5Y, N2A, mouse brain tissue, rat brain tissue, mouse cerebellum tissue.

Subcellular location: Membrane, Melanosome.

Database links: SwissProt: P09543 Human | P16330 Mouse | P13233 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50-1:200
IHC-P	1:500
FC	1:50-1:100
IHC-Fr	1:500
IF-Tissue	1:100

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

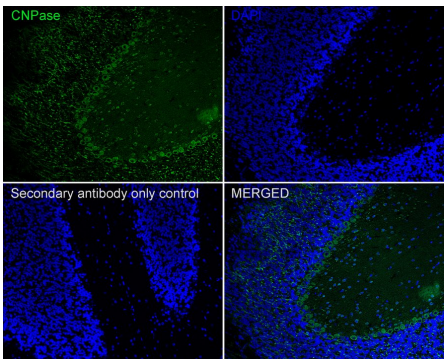

Fig1: Immunofluorescence analysis of frozen mouse cerebellum tissue with Rabbit anti-CNPase antibody (ET1702-46) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1702-46, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

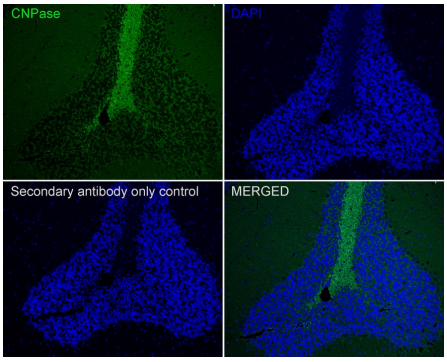

Fig2: Immunofluorescence analysis of paraffin-embedded mouse cerebellum tissue labeling CNPase with Rabbit anti-CNPase antibody (ET1702-46) at 1/100 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for 2 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1702-46, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

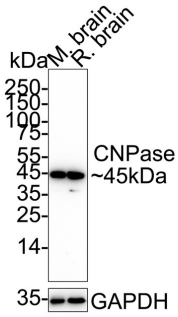

Fig3: Western blot analysis of CNPase on different lysates with Rabbit anti-CNPase antibody (ET1702-46) at 1/2,000 dilution.

Lane 1: Mouse brain tissue lysate
Lane 2: Rat brain tissue lysate

Lysates/proteins at 40 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 45 kDa

Exposure time: 46 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1702-46) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

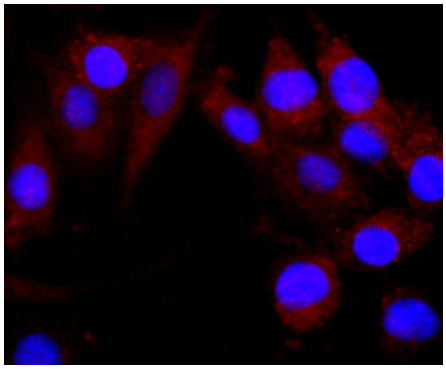

Fig4: ICC staining of CNPase in SHG-44 cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-46, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

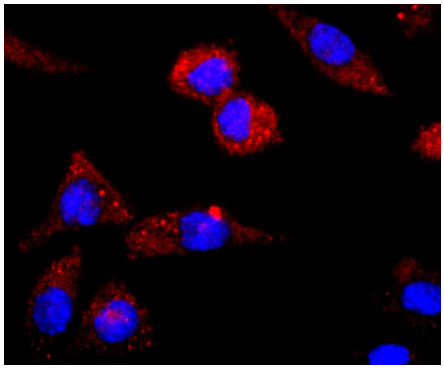

Fig5: ICC staining of CNPase in SH-SY5Y cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-46, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

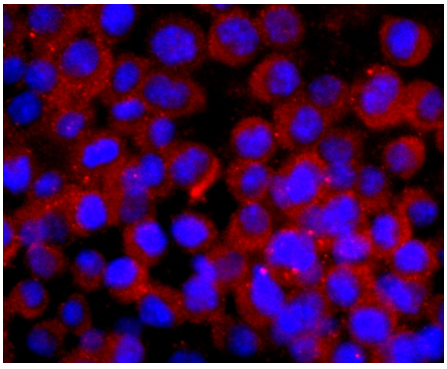

Fig6: ICC staining of CNPase in N2A cells (red). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1702-46, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®594 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

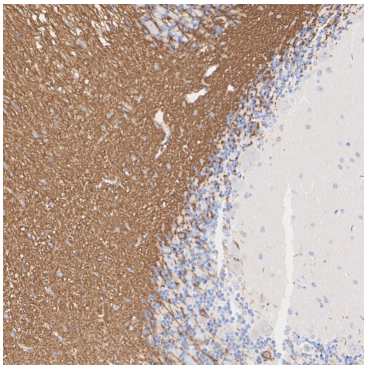

Fig7: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-CNPase antibody (ET1702-46) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-46) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

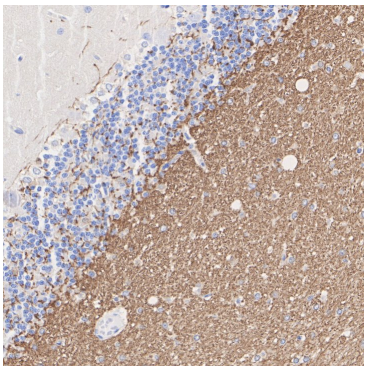

Fig8: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-CNPase antibody (ET1702-46) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1702-46) at 1/500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

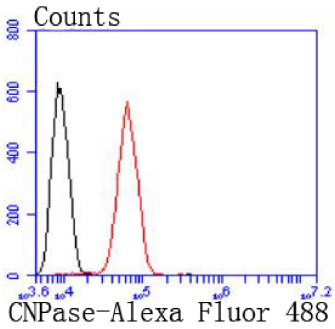

Fig9: Flow cytometric analysis of CNPase was done on SH-SY5Y cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1702-46, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Chang S et al. Neuropsin Inactivation Has Protective Effects against Depressive-Like Behaviours and Memory Impairment Induced by Chronic Stress. PLoS Genet 12:e1006356 (2016).
2. Kuzdas-Wood D et al. Involvement of Peripheral Nerves in the Transgenic PLP-a-Syn Model of Multiple System Atrophy: Extending the Phenotype. PLoS One 10:e0136575 (2015).