

Anti-Choline Acetyltransferase Antibody [JA67-11] ET1704-16



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IP, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 83 kDa
Clone number:	JA67-11

Description: Choline acetyltransferase (also designated choactase, choline O-acetyltransferase) synthesizes acetylcholine in cholinergic neurons. Multiple choactase mRNAs with different 5'-noncoding regions are expressed as R-, N1, N2-, S- and M-types. N1-, N2- and R-type mRNAs produce a single short enzyme, while M-type mRNA produces both long and short enzymes. The long enzyme is targeted to the nuclei of cells, whereas the short protein is found in cytoplasm. A novel NFkB binding site is located within the nerve growth factor-responsive enhancer element that is recognized by the NFkB protein p49, but not p65 or p50. Decreased choactase expression and increased NFkB activity are associated with aging and Alzheimer's disease, indicating that p49 is a negative regulator of choactase expression and suggesting a possible mechanism for aging-associated declines in cholinergic function. Phosphorylation of choactase has been shown to enhance choactase catalytic activity. Specifically, Serine 440 is found to be the phosphorylation site in a recombinant human short choactase by protein kinase C and is involved in regulation of the enzyme catalytic activity and binding to subcellular membranes.

Immunogen: Synthetic peptide within Human Choline Acetyltransferase aa 699-748 / 748.

Positive control: Human brain tissue lysate, mouse brain tissue lysate, rat brain tissue lysate, Hela, N2A, SH-SY5Y, mouse brain tissue, rat stomach tissue, mouse hippocampus tissue, mouse cerebral cortex tissue.

Subcellular location: Cytosol, nucleus, cytoplasm, neuron projection, presynapse

Database links: SwissProt: P28329 Human | Q03059 Mouse | P32738 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:50-1:200
IHC-P	1:50-1:200
FC	1:1,000
IP	Use at an assay dependent concentration.

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

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

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

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Images

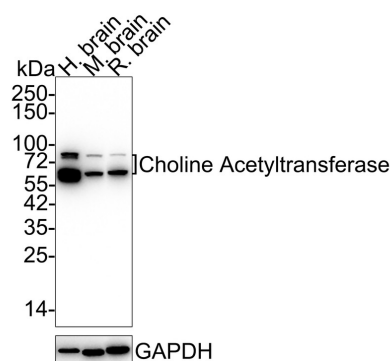


Fig1: Western blot analysis of Choline Acetyltransferase on different lysates with Rabbit anti-Choline Acetyltransferase antibody (ET1704-16) at 1/5,000 dilution.

Lane 1: Human brain tissue lysate

Lane 2: Mouse brain tissue lysate

Lane 3: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 83 kDa

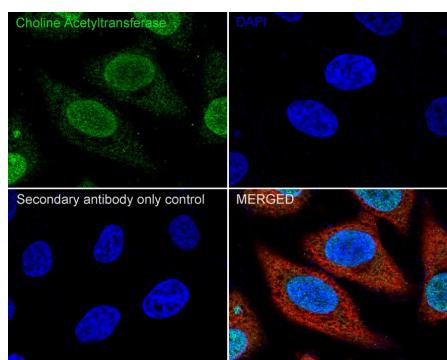
Observed band size: 60-83 kDa

Exposure time: 20 seconds;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDN/TBST for 1 hour at room temperature. The primary antibody (ET1704-16) at 1/5,000 dilution was used in 5% NFDN/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of HeLa cells labeling Choline Acetyltransferase with Rabbit anti-Choline Acetyltransferase antibody (ET1704-16) at 1/100 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Choline Acetyltransferase antibody (ET1704-16) at 1/100 dilution in 1% BSA in PBST overnight at 4°C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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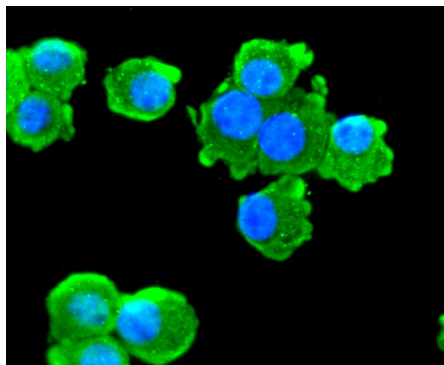


Fig3: ICC staining of Choline Acetyltransferase in N2A cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-16, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

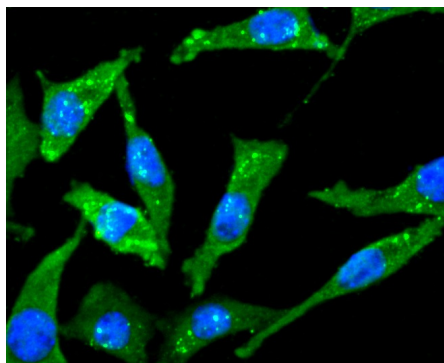


Fig4: ICC staining of Choline Acetyltransferase in SH-SY5Y cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1704-16, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

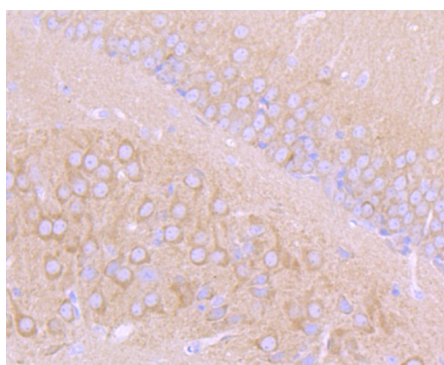


Fig5: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-Choline Acetyltransferase antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1704-16, 1/50) for 30 minutes 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.

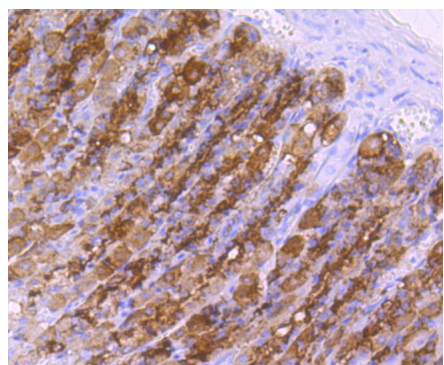


Fig6: Immunohistochemical analysis of paraffin-embedded rat stomach tissue using anti-Choline Acetyltransferase antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ET1704-16, 1/50) for 30 minutes 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.

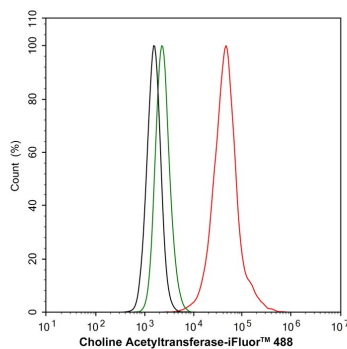


Fig7: Flow cytometric analysis of HeLa cells labeling Choline Acetyltransferase.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1704-16, 1µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. 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. Yu B et al. Mechanisms Underlying Footshock and Psychological Stress-Induced Abrupt Awakening From Posttraumatic "Nightmares". *Int J Neuropsychopharmacol* N/A:N/A (2015).
2. Hadzipasic M et al. Selective degeneration of a physiological subtype of spinal motor neuron in mice with SOD1-linked ALS. *Proc Natl Acad Sci U S A* 111:16883-8 (2014).

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