Anti-Choline Acetyltransferase Antibody [JA67-11] - BSA and Azide free

HA750406

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IP, IF-Cell, 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: SH-SY5Y cell lysate, Neuro-2a cell lysate, Mouse brain tissue lysate, Mouse cerebellum tissue lysate, Rat brain tissue lysate, Rat cerebellum tissue lysate, HeLa.

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

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

Storage Buffer: 1*PBS (pH7.4).

Storage Instruction: Store at 2-8℃. Avoid freeze.

Purity: Protein A affinity purified.

Images

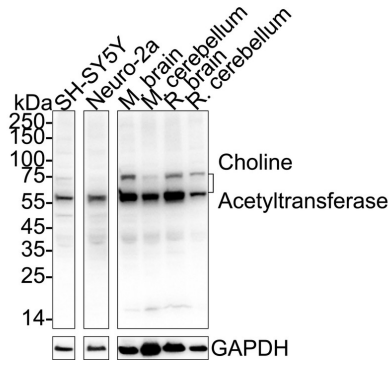

Fig1: This data was developed using ET1704-16, the same antibody clone in a different buffer formulation.
Western blot analysis of Choline Acetyltransferase on different lysates with Rabbit anti-Choline Acetyltransferase antibody (ET1704-16) at 1/5,000 dilution.

Lane 1: SH-SY5Y cell lysate (20 µg/Lane)
Lane 2: Neuro-2a cell lysate (negative) (20 µg/Lane)
Lane 3: Mouse brain tissue lysate (40 µg/Lane)
Lane 4: Mouse cerebellum tissue lysate (40 µg/Lane)
Lane 5: Rat brain tissue lysate (40 µg/Lane)
Lane 6: Rat cerebellum tissue lysate (40 µg/Lane)

Predicted band size: 83 kDa
Observed band size: 50/75 kDa

Exposure time: 5 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 (ET1704-16) at 1/5,000 dilution was used in primary antibody dilution (K1803) at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

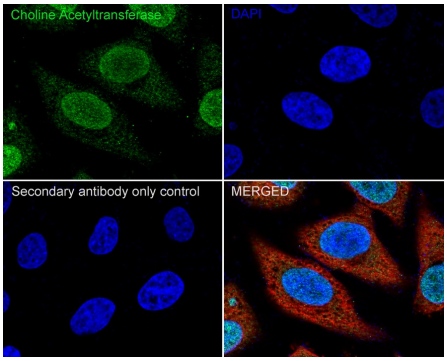

Fig2: This data was developed using ET1704-16, the same antibody clone in a different buffer formulation.
Immunocytochemistry analysis of HeLa cells labeling Choline Acetyltransferase with Rabbit anti-Choline Acetyltransferase antibody (ET1704-16) at 1/100 dilution.

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 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

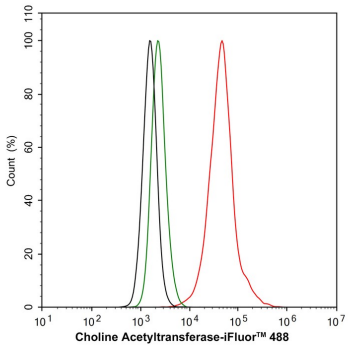

Fig3: This data was developed using ET1704-16, the same antibody clone in a different buffer formulation.
Flow cytometric analysis of HeLa cells labeling Choline Acetyltransferase.

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℃ 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℃. 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. Gabalski AH et al. Circulating extracellular choline acetyltransferase regulates inflammation. J Intern Med. 2024 Mar
2. Liu J et al. Choline acetyltransferase and vesicular acetylcholine transporter are required for metamorphosis, reproduction, and insecticide susceptibility in Tribolium castaneum. Gene. 2022 Oct