APB929Mu01 100μg
Active Choline Acetyltransferase (ChAT)
Organism Species: *Mus musculus (Mouse)*
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Met1~Ser641
Tags: His and TrxA Tag
Purity: >90%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 0.01% Sarcosyl, 5%Trehalose .
Original Concentration: 200μg/mL
Applications: Activity Assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 7.8
Predicted Molecular Mass: 89.8kDa
Accurate Molecular Mass: 60&70kDa as determined by SDS-PAGE reducing conditions.
Phenomenon explanation:
The possible reasons that the actual band size differs from the predicted are as follows:
1. Splice variants: Alternative splicing may create different sized proteins from the same gene.
2. Relative charge: The composition of amino acids may affects the charge of the protein.
3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.
4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.
5. Polymerization of the target protein: Dimerization, multimerization etc.

[USAGE]

Reconstitute in 10mM PBS (pH7.4) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8ºC for one month.

Aliquot and store at -80ºC for 12 months.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37ºC for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[SEQUENCE]

MPILEKVPPKMPVQASSCEEVLDLPKLPVPPLQQTLATYLQCMQHLVPEEQFRKSQAIVKRFGAPGGLGETLQEKLLERQEKTANWVSEY
WLNDMYLNNRLALPVNSSPAVIFARQHFQDTNDQLRFAASLISGVLSYKALLDSQSIPTDWAKGQLSGQPLCMKQYYRLFSSYRLPGHTQ
DTLVAQKSSIMPEPEHVIVACCNQFFVLDVVINFRRLSEGDLFTQLRKIVKMASNEDERLPPIGLLTSDGRSEWAKARTVLLKDSTNRDS
LDMIERCICLVCLDGPGTGDLSDTHRALQLLHGGGCSLNGANRWYDKSLQFVVGRDGTCGVVCEHSPFDGIVLVQCTEHLLKHMMTGNKK
LVRVDSVSELPAPRRLRWKCSPETQGHLASSAEKLQRIVKNLDFIVYKFDNYGKTFIKKQKCSPDGFIQVALQLAYYRLYQRLVPTYESA
SIRRFQEGRVDNIRSATPEALAFVQAMTDHKAAVLASEKLQLLQRAIQAQTEYTVMAITGMAIDNHLLALRELARDLCKEPPEMFMDETY
LMSNRFILSTSQVPTTMEMFCCYGPVVPNGYGACYNPHAEAITFCISSFHGCKETSSVEFAEAVGASLVDMRDLCSSRQPADSKPPTAKE
RARGPSQAKQS

[ACTIVITY]

Choline Acetyltransferase (ChAT) is the enzyme responsible for synthesizing acetylcholine (ACh) from acetyl-CoA and choline in presynaptic neurons. It is crucial for cholinergic neurotransmission, influencing cognitive functions, muscle activation, and autonomic processes. ChAT is primarily localized in nerve terminals and is a biomarker for cholinergic neurons.ChAT synthesizes acetylcholine, while AChE breaks it down. They work in tandem to maintain the appropriate level of acetylcholine in the synaptic cleft for proper neural signaling.Thus a functional ELISA assay was conducted to detect the interaction of recombinant mouse ChAT and recombinant bovine ACHE. Briefly, ChAT was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl

were then transferred to ACHE-coated microtiter wells and incubated for 1h at 37℃. Wells were washed with PBST and incubated for 1h with anti-ChAT pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37℃, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50 μL stop solution to the wells and read at 450/630nm immediately. The binding activity of recombinant mouse ChAT and recombinant bovine ACHE was shown in Figure 1, the EC50 for this effect is 0.115ug/mL.

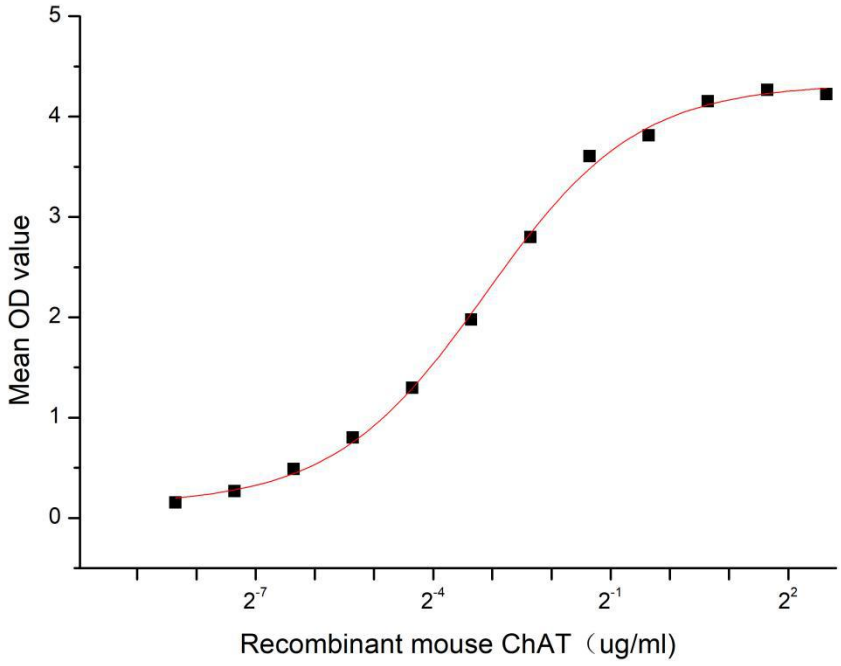

Figure 1. The binding activity of recombinant mouse ChAT and recombinant bovine ACHE

[IDENTIFICATION]

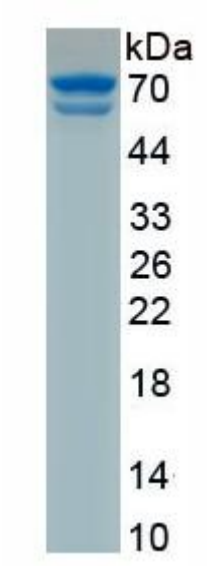

Figure 2. SDS-PAGE

Sample: Active recombinant ChAT, Mouse

[IMPORTANT NOTE]

The kit is designed for research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.