



**PRODUCT INFORMATION &  
MANUAL**

**Choline Acetyltransferase/  
ChAT Activity Assay Kit  
(Colorimetric)  
*NBP3-24479***

For research use only.  
Not for diagnostic or therapeutic  
procedures.

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## Choline Acetyltransferase/ChAT Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24479

Method: Colorimetric method

Specification: 96T (Can detect 48 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 1.21 U/g fresh weight

Detection range: 1.21-40 U/g fresh weight

Average intra-assay CV (%): 5.1

Average inter-assay CV (%): 9.4

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

## General information

### ▲ Intended use

This kit can be used to measure choline acetyltransferase (ChAT) activity in animal tissues samples.

### ▲ Background

Choline Acetyltransferase (ChAT, EC 2.3.1.6) is an enzyme for biosynthesis of acetylcholine, which generates acetylcholine by catalyzing acetyl group transfer from acetyl coenzyme A to choline. ChAT is synthesized in the perinuclear body of neurons and transported to neurons through the mechanism of slow axon transport. ChAT is the most specific indicator for monitoring the functional status of cholinergic neurons in central and peripheral nervous systems.

### ▲ Detection principle

Acetyl-CoA can react with choline under the catalysis of choline acetyltransferase (ChAT) to produce coenzyme A (CoA), CoA can combine with the 4, 4-dithiopyridine. The activity of ChAT can be calculated indirectly by measuring the OD value at 324 nm.

## ▲ Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	26 mL × 1 vial	2-8°C , 12 months
Reagent 2	Inhibitor	1.2 mL × 1 vial	-20°C , 12 months
Reagent 3	Substrate A	Powder × 1 vial	-20°C , 12 months
Reagent 4	Substrate B	1.2 mL × 2 vials	2-8°C , 12 months
Reagent 5	Accelerant A	3 mL × 1 vial	2-8°C , 12 months
Reagent 6	Accelerant B	1.2 mL × 2 vials	-20°C , 12 months
Reagent 7	Chromogenic Agent	2 mL × 1 vial	2-8°C , 12 months
	UV Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

## ▲ Materials prepared by users

### Instruments

Microplate reader (324 nm), Micropipettor, Centrifuge, Incubator, Water bath, Vortex mixer

### Reagents

Double distilled water, Normal saline (0.9% NaCl), PBS (0.01 M, pH 7.4)

## ▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

## ▲ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

## Pre-assay preparation

### ▲ Reagent preparation

The preparation of reagent 3 working solution:

Dissolve a vial of powder with 2.4 mL of double distilled water fully. The prepared solution can be stored at  $-20^{\circ}\text{C}$  for 3 months. It is recommended to aliquot reagent 3 working solution into smaller quantities and store at  $-20^{\circ}\text{C}$ .

### ▲ Sample preparation

Preparation of 20% tissue homogenate

Take 0.1-1 g tissue sample, wash with PBS (0.01 M, pH 7.4) at  $2-8^{\circ}\text{C}$ . Absorb the water with filter paper and weigh. Then add 4 times the volume of PBS (0.01 M, pH 7.4) according to the ratio of Weight (g): Volume (mL) =1:4. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

### ▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.21-40 U/g wet weight).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
20% Mouse brain tissue homogenate	1-2
20% Rat heart tissue homogenate	1
20% Rat liver tissue homogenate	1
20% Mouse kidney tissue homogenate	1
20% Rat lung tissue homogenate	1
20% Rat heart tissue homogenate	1

**Note:** The diluent is normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4).

# Assay protocol

## ▲ Plate set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S1'	S9	S9'	S17	S17'	S25	S25'	S33	S33'	S41	S41'
B	S2	S2'	S10	S10'	S18	S18'	S26	S26'	S34	S34'	S42	S42'
C	S3	S3'	S11	S11'	S19	S19'	S27	S27'	S35	S35'	S43	S43'
D	S4	S4'	S12	S12'	S20	S20'	S28	S28'	S36	S36'	S44	S44'
E	S5	S5'	S13	S13'	S21	S21'	S29	S29'	S37	S37'	S45	S45'
F	S6	S6'	S14	S14'	S22	S22'	S30	S30'	S38	S38'	S46	S46'
G	S7	S7'	S15	S15'	S23	S23'	S31	S31'	S39	S39'	S47	S47'
H	S8	S8'	S16	S16'	S24	S24'	S32	S32'	S40	S40'	S48	S48'

Note: S1-S48, sample wells; S1'- S48', control wells.



# Assay protocol

## ▲ Detailed operation steps

1. Preparation of substrate working solution:

(the prepared substrate working solution must be use out in 3 hours)

	Substrate working solution
Reagent 1 ( $\mu\text{L}$ )	$210 \times (2n+2^*)$
Reagent 2 ( $\mu\text{L}$ )	$10 \times (2n+2^*)$
Reagent 3 working solution ( $\mu\text{L}$ )	$20 \times (2n+2^*)$
Reagent 4 ( $\mu\text{L}$ )	$20 \times (2n+2^*)$
Reagent 5 ( $\mu\text{L}$ )	$20 \times (2n+2^*)$
Reagent 6 ( $\mu\text{L}$ )	$20 \times (2n+2^*)$
Total amount of mixture reagent ( $\mu\text{L}$ )	$300 \times (n+2^*)$

**Note:**

n refers to the number of sample.

2\*: Prepare 2 more tubes of substrate working solution.

## 2. Operation procedure

### 1) Pretreatment

- (1) **Control tube:** add 50  $\mu\text{L}$  of sample into 2 mL EP tube, then incubate in 100°C water bath for 2 min.  
**Sample tube:** add nothing.
- (2) Add 300  $\mu\text{L}$  of substrate working solution (preheated for 5 min) to each tube.
- (3) **Control tube:** add nothing.  
**Sample tube:** add 50  $\mu\text{L}$  of sample.
- (4) Mix fully and incubate at 37°C water bath for 20 min, then incubate in 100°C water bath for 2 min to stop the reaction.
- (5) Add 850  $\mu\text{L}$  of double distilled water to each tube.
- (6) Mix fully and centrifuge at 3100 g for 10 min, then take 750  $\mu\text{L}$  of supernatant to the new corresponding 2 mL EP tube for chromogenic reaction.

### 2) Chromogenic reaction

- (1) Add 15  $\mu\text{L}$  of reagent 7 to each tube.
- (2) Mix fully and stand at room temperature for 15 min. Take 250  $\mu\text{L}$  of supernatant to the corresponding wells of microplate and measure the OD value of each well at 324 nm.

## ▲ Summary operation table

### 1.Pretreatment

	Sample tube	Control tube
Sample ( $\mu\text{L}$ )		50
Incubate in 100°C water bath for 2 min		
Substrate working solution ( $\mu\text{L}$ ) (preheated for 5 min)	300	300
Sample ( $\mu\text{L}$ )	50	
Mix fully and incubate at 37°C water bath for 20 min, then incubate in 100°C water bath for 2 min to stop the reaction.		
Double distilled water ( $\mu\text{L}$ )	850	850
Mix fully and centrifuge at 3100 g for 10 min, then take 750 $\mu\text{L}$ of supernatant to the new corresponding 2 mL EP tube for chromogenic reaction.		

### 2.Chromogenic reaction

	Sample tube	Control tube
Supernatant ( $\mu\text{L}$ )	750	750
Reagent 7 ( $\mu\text{L}$ )	15	15
Mix fully and stand at room temperature for 15 min. Take 250 $\mu\text{L}$ of supernatant to the corresponding wells of microplate and measure the OD value of each well at 324 nm.		

## ▲ Calculation

### Unit definition:

The ability of transferring 1 nmol acetyl to choline by 1 g of wet weight tissue at 37°C and pH 7.2 is defined as 1 unit.

### Calculation formula

$$\text{ChAT activity} \frac{\text{U/g wet weight}}{\text{U/g wet weight}} = \frac{A_2 - A_1}{t \times \epsilon \times d} \times \frac{V_2}{V_1} \div \frac{m}{V_3}$$

### Note:

A<sub>1</sub>: the OD value of control

A<sub>2</sub>: the OD value of sample

t: the time of enzymatic reaction, 20 min.

ε: 1.98×10<sup>-5</sup> L/(nmol·cm), the molar extinction coefficient of product at 324 nm.

d: the optical path of cuvette, 0.7 cm.

V<sub>1</sub>: the volume of sample, 50 μL.

V<sub>2</sub>: the total volume of reaction, 1200 μL.

V<sub>3</sub>: the volume of PBS added in sample preparation step, L.

m: the weight of sample in sample preparation step, g.

## Appendix I Data

### ▲ Example analysis

Take 50  $\mu$ L of 20% mouse heart tissue homogenate sample and carry the assay according to the operation table. The results are as follows:

The average OD value of the sample is 0.085, the average OD value of the control is 0.078, and the calculation result is:

$$\text{ChAT activity (U/g wet weight)} = (0.085 - 0.078) \div 20 \div 1.98 \div 0.7 \times 100000 \times 1200 \div 50 \div 0.2 \times 0.8 \div 1000 = 2.42 \text{ U/g wet weight}$$

## Appendix II References

1. Tuček S. Regulation of Acetylcholine Synthesis in the Brain [J]. Journal of Neurochemistry, 2010, 44(1): 11-24.
2. Oda Y, Nakanishi I. The distribution of cholinergic neurons in the human central nervous system[J]. Histology & Histopathology, 2000, 15(3): 825-834.