Choline Acetyltransferase (ChAT) Assay Kit

(Cat/No.:BC145 Size:40T/20S)

1. Principle of Measurement

The measurement of ChAT activity is based on the reaction catalyzed by ChAT with Acetyl Coenzyme A and choline in presence as the substrate. The product of the reaction would further react with chromogenic agent and from the absorbance values measured at 324 nm, the ChAT activity can be calculated.

2. Compositions and Preparation (The kit is valid for 3 months)

Reagent I: 1 Bottle×6ml. Preserved at 4°C.

Note that crystallization may occur at low temperature and thus, the solution should be warmed at 37°C till clear before the measurement.

Reagent II: 1 Bottle of Powder.

Preparation of Reagent II Solution: Dissolve the powder with 220μl distilled water. The excess amount can be preserved at -20°C.

Reagent III: 1 Bottle of Powder

Preparation of Reagent III Solution: Dissolve the powder with 440μl double distilled water (DDW) prior to the measurement. The excess amount can be preserved at 4°C.

Reagent IV: 2 Bottle of Liquid.

Preparation of Reagent IV Solution: Dilute the liquid with DDW with the ratio of 1:1 (v/v). The excess amount can be preserved at 4°C.

Reagent V: 1 Bottle of Liquid. Preserved at 4°C.

Reagent VI: 1 Bottle of Liquid. Preserved at 4°C.

Reagent VII: 1 Bottle of Liquid. Preserved at 4°C.

3. Required instruments and reagents

A tunable 324nm ultraviolet spectrophotometer and a 1cm optical path (2mm inner diameter) quartz cuvette, vortex mixer, 37℃ water bath (or constant temperature box), ice water bath, centrifuge, double distilled water (or distilled water), protein determination reagent (optional when calculating protein concentration, the reagent kit is available for purchase from our company).

4. Procedures

I. Pre-Treatment

Weigh the sample precisely and add physiological saline with the ratio of 1:9 (g/ml). Homogenize the mixture and centrifuge the homogenate at 2,500 rpm for 10 min. Extract the supernatant to half its original concentration with saline. Boil enough amount of solution for the reference measurement.

II. Procedures

Compositions (μl)	Sample	Reference
Reagent I	105	105
Reagent II	5	5
Reagent III	10	10
Reagent IV	10	10
Reagent V	10	10
Reagent VI	10	10
Mix and warm at 37°C for 5 min.		
Pre-Treated Solution	25	25 (Boiled)
Mix and warm at 37°C for 20 min and then boil the solution for 2 min to terminate the reaction.		
Distilled Water	425	425
Mix and centrifuge at 4,000 rpm for 10 min and extract the supernatant.		
Supernatant	500	500
Reagent VII	10	10

Mix and set aside for 15 min. Regulate the spectrophotometer and clean the 2mm i.d. quartz cuvette with 1cm path length with distilled water. Record the optical density (OD) values of each tube.

I. Definition

One choline acetyltransferase activity unit is defined as 1 μmol acetyl group transferred to choline per minute at 37°C with pH=7.2.

II. Formula

$$\underset{U/g}{ChAT\ Activity} = \frac{OD_{Sample} - OD_{Reference}}{MAC^*} \div \frac{Time}{20\,min} \div \frac{Path\ Length}{1cm} \times \frac{V_{Total}(600\mu l)}{V_{Sample}(25\mu l)} \div \underset{g/ml}{C_{Tissue}}$$

Note *: MAE represents the millimolar attenuation coefficient and is 1.98 $\times 10^{-2}$ $mM^{-1}\cdot cm^{-1}$in this case.

III. Example

5% rat brain tissue homogenate was measured and the OD values were 0.354 and 0.260 respectively.

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$$= \frac{0.354 - 0.260}{1.98 \times 10^{-2}} \div 20 \div 1 \times \frac{600}{25} \div \frac{5\%}{1 - 5\%} = = 102.55\text{U/g}$$

5. Significance

Acetylcholine (Ach) acts as an excitatory neurotransmitter for the adjustment of smooth muscle within esophagus. The contraction of smooth muscles are based on the releasing of acetylcholine. Choline acetyltransferases and acetylcholinesterases are the Ach synthase and hydrolase
respectively and are responsible for the adjustment of Ach metabolism. Because of this, the activities of the two enzymes can represent the status of endogenous cholinergic nerve function in esophagus.

Ach is the main neurotransmitter in many kinds of neurons and is generated with choline and acetyl-coenzyme A from mitochondria with choline acetyltransferase in presence.generation, the Ach is released and stimulate specific receptors and then the interaction soon be terminated with Cholinesterase which catalyze the decomposition of acetylcholine into choline and acetate. The concentration of acetylcholine is monitored with choline acetyltransferase and choline taken.