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Acetylcholinesterase Activity Colorimetric Assay Kit

rev. 07/17

(Catalog # K764-100; 100 assays; Store at -20°C)

I. Introduction:

Acetylcholinesterase (AChE) is one of the most crucial enzymes associated with nerve response and function. AChE is a serine protease that hydrolyzes the neurotransmitter acetylcholine. Irreversible inhibition of AChE may lead to muscular paralysis, convulsions, bronchial constriction, and death by asphyxiation. BioVision's Acetylcholinesterase Activity Assay Kit provides a simple and sensitive method for continuous monitoring of enzymatic activity using colorimetry (OD 570 nm). In this assay, AChE converts acetylcholine substrate to choline, which is then oxidized by choline oxidase (CO) to produce an intermediate. The intermediate reacts with a highly specific probe to generate color. This assay kit can detect acetylcholinesterase activity as low as 0.5 mU/ml in a variety of samples.

II. Application:

- Measurement of AChE activity in various tissues/cells
- Screening of AChE inhibitors

III. Sample Type:

- Serum, plasma or blood.
- Animal tissues: liver, heart, kidney, etc.
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	K764-100	Cap Code	Part Number
AChE Assay Buffer	25 ml	WM	K764-100-1
AChE Probe (DMSO)	200 µl	Red	K764-100-2A
AChE Substrate (Lyophilized)	1 vial	Blue	K764-100-3
Choline Oxidase Enzyme Mix (Lyophilized)	1 vial	Green	K764-100-4
Choline Standard (Lyophilized)	1 vial	Yellow	K764-100-5
AChE Positive Control (Lyophilized)	1 vial	Orange	K764-100-6

V. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer

VI. Storage and Handling:

Store kit at -20°C, protected from light. Warm Assay Buffer to room temperature before use. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **AChE Probe:** Store at -20°C. Warm to room temperature before use. Use within two months.
- **AChE Substrate:** Reconstitute with 110 µl DI Water to generate 50 mM solution. Store at -20°C. Use within two months. Keep on ice while in use.
- **Choline Oxidase Enzyme Mix:** Reconstitute with 220 µl AChE Assay Buffer. Aliquot and store at -20°C. Stable for 2 months.
- **Choline Standard:** Reconstitute with 100 µl AchE Assay Buffer to generate 50 mM stock Choline Standard Solution. Use within 2 months.
- **AChE Positive Control:** Reconstitute with 100 µl of AChE Assay Buffer. Aliquot and store at -20°C. Use within two months.

VIII. Acetylcholinesterase Assay Protocol:

1. **Sample Preparation:** Homogenize 25 mg of sample (wet weight or cell pellet) in extraction buffer of your choice (we recommend the use of EZLys™ Tissue Protein Extraction Reagent [Cat. # 8002] or EZLys™ Mammalian Protein Extraction Reagent [Cat. # 8004]). Centrifuge at 10,000 X g for 5 min. Collect the supernatant. Add 1-5 µl of supernatant into a 96-well plate and adjust the volume to 50 µl with AChE Assay Buffer. Serum, plasma or blood (1-5 µl) can be tested directly.

   Notes:

   a: For unknown samples, we suggest testing several doses to ensure the reading are within the Standard Curve range.

   b: For samples having background, prepare parallel well(s) containing the same amount of sample as in the test well. Adjust the volume to 50 µl with AChE Assay Buffer.

   c. Extraction buffers containing 1% or more of Triton X-100 will interfere with the assay.

2. **Standard Curve Preparation:** Dilute Choline Standard to 0.5 mM by adding 10 µl of 50 mM Choline Standard into 990 µl of AChE Assay Buffer, mix well. Add 0, 2, 4, 6, 8 and 10 µl of the diluted 0.5 mM Choline Standard into a series of wells in 96-well plate. Adjust the volume to 50 µl/well with the AChE Assay Buffer to generate 0, 1, 2, 3, 4 and 5 nmol/well of Choline Standard.
3. **AChE Positive Control:** Dilute positive control solution 1:10 in AChE Assay Buffer. Add 1-10 µl of diluted Positive Control into desired wells(s) and adjust the final volume to 50 µl with AChE Assay Buffer.
4. **Reaction Mix:** Mix enough reagents for the number of assays (samples, Standards & Positive Control) to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	*Background Control Mix
AChE Assay Buffer	45 µl	46 µl
Choline Oxidase Enzyme Mix	2 µl	2 µl
AChE Probe	2 µl	2 µl
AChE Substrate	1 µl	----

Mix well. Add 50 µl of Reaction Mix to each well containing the Choline Standards, Positive Control and samples. Mix well.

* For samples having high background, add 50 µl of Background Control Mix to sample background control well(s). Mix well.

5. **Measurement:** Incubate for 20-30 min. at 37°C and measure absorbance (OD 570 nm).

   Note: Incubation time depends on the AChE Activity in the samples. We recommend measuring OD in a kinetic mode, and choosing two time points (T_1 and T_2) in the linear range to calculate the AChE activity of the samples. The Standard Curve can be read in end point mode (i.e. at the end of incubation time).

6. **Calculations:** Subtract 0 Choline Standard reading from all readings. Plot the Choline Standard Curve. If sample background control reading is significant, subtract background control reading from sample readings. Calculate the AChE activity of the test sample: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the Choline Standard Curve to get B nmol of Choline generated by AChE during the reaction time ($\Delta T = T_2 - T_1$).

Sample AChE Activity = B/(ΔT X V) x D = nmol/min/ml = mU/ml

Where: **B** is the Choline amount from the Standard Curve (nmol)

ΔT is the reaction time (min.)

V is the sample volume added into the reaction well (ml)

D is the sample dilution factor

Unit Definition: One milliunit of AChE activity is the amount of enzyme that will generate 1.0 nmol of Choline per min. at pH 7.4 at 37°C.

(a) (b)

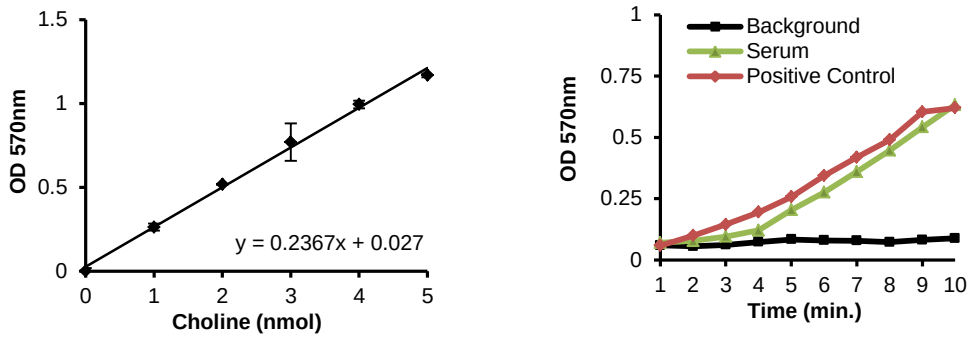

Figure: Choline Standard Curve (a). AChE activity in serum (1 µl) & Positive Control (1 µl). Assays were performed following the kit protocol.

IX RELATED PRODUCTS:

Choline/Acetylcholine Quantification Colorimetric/Fluorometric Kit (K615)
Phosphatidylcholine Colorimetric/Fluorometric Kit (K576)
EZLys™ Tissue Protein Extraction Reagent (8002)
EZLys™ Mammalian Protein Extraction Reagent (8004)

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