EZ- Acetylcholinesterase Assay Kit

Metabolism Assay Kit

(Colorimetric)

Cat. No. DG-ACE100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

Acetylcholinesterase (AChE) is a serine protease that hydrolyzes the neurotransmitter acetylcholine, an enzyme that is important for nerve responses and functions. Irreversible inhibitors of AChE can cause muscle paralysis, convulsions, bronchial constriction, and death by asphyxiation.

The EZ-Acetylcholinesterase Activity Assay Kit provides a simple, sensitive, colorimetric method for monitoring enzyme activity. In this assay, AChE converts the substrate acetylcholine to choline, which is then oxidized by choline oxidase (CO) to produce an intermediate (H_2O_2) that induces color development in the probe.

▪ Kit Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage (reconstituted)
AChE Assay buffer	25㎖	-	-20 ℃, 2 Month
AChE Enzyme mix (Lyophilized)	1 vial	Red	-20 ℃, 2 Month
AChE Substrate	5 vial	Blue	-20 ℃, 2 Month
AChE Probe	200㎕	Yellow	-20 ℃, 2 Month
AChE Standard (50mM)	100㎕	Green	-20 ℃, 2 Month

* This product is intended for research purposes only and is not intended for human or diagnostic use.

* Regarding the number of tests that can be performed with this product, 100 assays means that reagents are provided that can process 100 wells based on 1 well of a microplate. Considering sample duplication, etc. along with wells processed as standard wells, reagent blanks, sample blanks, etc., the actual number of samples that can be processed is in the range of 20 to 40 samples. Please review the product description carefully and determine the number of kits required based on the characteristics of the sample you wish to test.

▪ Sample type

- Serum, plasma, blood
- Animal tissues such as liver, heart, kidney, etc. and cultured cells

▪ Preparation of Reagent

Solution	Preparation	Storage and Stability
AChE Enzyme mix	Add 220 ㎕ AChE assay buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20℃.
AChE Substrate	Add 1 ml of DMSO and dissolve completely. *DMSO is not included in the kit.	Substrates dissolved in DMSO cannot be reused. Please melt and use a new one for each experiment.
AChE Probe	It is mixed in DMSO. Dissolve it sufficiently at room temperature before use.	AChE Probe is stable for 2 months at -20℃.
AChE Standard	Use after melting it sufficiently at room temperature.	The remaining solution can be stored at -20℃, but use within 2 months if possible.

* Use the assay buffer after sufficiently warming up to room temperature before the experiment.
* Using cold buffer may inhibit enzyme activity and affect the results.

▪ General Protocol

1. Standard preparation

Prepare a 0.5 mM AChE standard solution by mixing 10 ㎕ of 50 mM AChE standard and 990 ㎕ of assay buffer. Dispense 0, 2, 4, 6, 8, and 10 ㎕ of 0.5 mM AChE standard solution into a 96-well plate and adjust the final volume to 50 ㎕ with assay buffer.

Standard No.	Volume of 0.5mM AChE standard	Assay buffer	Final standard volume in well	Final standard amount in well (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	1
3	4 ㎕	46 ㎕	50 ㎕	2
4	6 ㎕	44 ㎕	50 ㎕	3
5	8 ㎕	42 ㎕	50 ㎕	4
6	10 ㎕	40 ㎕	50 ㎕	5

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.
* Standard recommends measurement during each experiment.

2. Sample preparation

Add 1-5 ㎕ of the prepared sample to a 96-well plate, and adjust the final volume to 50 ㎕ with assay buffer. (n≥2)

1) Extract 1-5 x 10^6 cells or 20 mg tissue sample using an appropriate extraction buffer. Since this is an experiment to analyze the activity of enzyme (AChE), a non-denaturing buffer must be used as the available extraction buffer. Alternatively, add cold 0.1 M phosphate buffer (pH 7.5) and homogenize on ice. The extracted sample is centrifuged at 10,000 x g for 5 minutes and the supernatant is recovered and used.

2) Serum, plasma or blood can be analyzed without pretreatment.

3) For unknown samples or samples being measured for the first time, it is recommended to conduct a preliminary experiment to ensure that the measured values are within the standard curve before use.

4) Buffers containing more than 1% Triton X-100 may interfere with the analysis.

5) If the measured value of the sample has a high background value, prepare the same amount of sample used for the measurement for a sample background control.

3. Reaction mixture preparation

This is the volume based on one well assay. Calculate the assay volume to be used in the experiment and prepare the reaction mix considering the loss volume.

Components	Reaction mixture	Background Reaction mixture
AChE Assay buffer	45㎕	46㎕
AChE Enzyme mix	2㎕	2㎕
AChE Probe	2㎕	2㎕
AChE Substrate	1㎕	-
Total	50㎕	50㎕

4. Add 50㎕ of the reaction mixture to each well containing the AChE standard and sample using a multi pipette, then mix well.

* If you have prepared a background control, add the background mixture to the background control well.

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5. After reacting the plate at 37℃ in a light-blocking condition for 30 minutes, gently shake it and measure the absorbance at a wavelength of 570 nm using a micro plate reader.

- The appropriate incubation time may vary depending on the activity of AchE in the sample. For accurate measurement, measure the OD value in Kinetic mode and select two time points (T1, T2. ΔT=T2-T1) within the range where the graph forms a straight line. (The OD values at this time are called A1 and A2. ΔOD=A2-A1).
- The standard curve is created in end point mode at T2 time point, and the ΔOD value is applied to calculate the amount of choline produced during that time. (**B** nmol).

▪ Calculation

1. Subtract the standard 1 value (blank) from all measurements.
   * Blank = OD_{570nm} from AChE standard #1 (0 nmol AChE)

2. Calculate the average of duplicate measurements for each standard well and sample well.

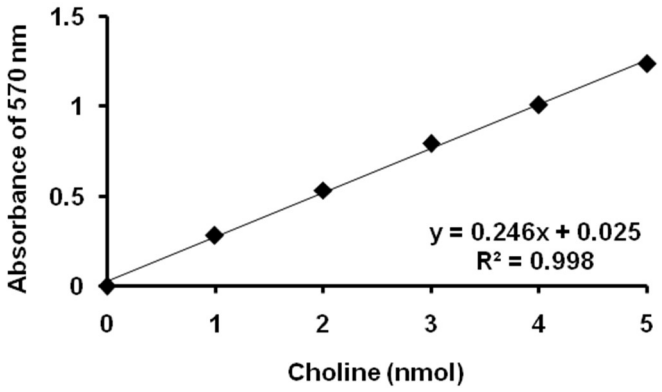
3. A standard curve is drawn using the AChE standard absorbance.
   (AChE standard vs OD 570 nm)

4. The amount of choline produced in the reaction is calculated by substituting the sample measurement values into the standard curve. (**B** nmol).
   * If background control is set, the amount of choline is calculated by subtracting the background control measurement value from the sample measurement value.

5. The activity of AchE is calculated using the time point (ΔT) obtained from the kinetic assay and the amount of Choline obtained above, as follows.

AChE activity = B/(ΔT x V) x D =nmol/min/㎖ =mU/㎖

B = Amount of choline obtained from the standard curve (nmol)
ΔT = Reaction time (T2-T1, min)
V = Sample volume (㎖)
D = Sample dilution ratio (if diluted 2-fold, calculate as x2, not x1/2)



Choline standard curve. Assay was performed following the kit protocol.

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▪ Related Product

	Products	Catalog No.	Assay
	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay

	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay