(FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS!)

Cu^{2+}-ATPase Assay Kit

Catalog No.: BC00195

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Cu^{2+}-ATPase Assay Kit
Detection Method	Colorimetric
Sample Type	Tissue
Assay Type	Quantitative
Detection Instrument	Spectrophotometer, Microplate reader (660 nm)

Product Introduction

ATPase is located on the cell membranes and organelle membranes of tissues, serving as a type of protease on biological membranes. It plays vital roles in substance transport, energy conversion and information transmission. A series of changes in ATPase activity occur under oxygen deficiency and certain disease conditions in the body. In addition, some genetic diseases are also related to the level of this enzyme activity.

Principle

ATPase can decompose ATP into ADP and inorganic phosphorus. The activity of ATPase can be determined by measuring the amount of inorganic phosphorus. Cu^{2+}-ATPase is an enzyme that can be activated by potassium and inhibited by vanadate.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	10 mL×2	Store at 4°C
Reagent 2	Liquid	10 mL×1	Store at 4°C
Reagent 3	Liquid	7 mL×1	Store at 4°C

Reagent 4	Powder	3 vials	Store at 4°C. Add 5 mL of double-distilled water to each powder vial and dissolve completely after use. After preparation, store below -20 °C for up to 7 days. Do not repeat freeze-thaw cycles.
Reagent 5	Powder	1 vial	Store at 4°C. Add 5 mL of double-distilled water to each vial and dissolve completely (moderate heating is acceptable) before use.
Reagent 6	Powder	1 vial	Store at 4°C. Pour the powder into the solvent and dissolve completely before use.
	Liquid	3 mL×1	
Reagent 7	Liquid	10 mL×1	RT
Reagent 8	Powder	3 vials	Store at 4°C. Add 40 mL of double-distilled water to each bottle of powder to dissolve completely before use. Store at 4°C. Discard if the solution darkens in color.
Reagent 9	Powder	1 vial	Store at 4°C. Dissolve each bottle of powder with 100 mL of double-distilled water before use, and store at 4°C.
Reagent 10	2.5 mol/L Sulfuric Acid	100 mL×1	RT
Reagent 11	10 mmol/L Standard Phosphorus Stock Solution	10 mL×1	Store at 4°C. Dilute the stock solution with double-distilled water at a ratio of 1:19 before use (e.g., take 0.5 mL of the stock solution and dilute to 10 mL with double-distilled water) to prepare a 0.5 μmol/mL working standard phosphorus solution.
Consumable 1	Microplate	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Preparation of Phosphorus Determination Reagent: Prepare the reagent in the following ratio and adding order: Reagent 8: Reagent 9: H_2O: 2.5 mol/L sulfuric acid = 1:1:2:1. The prepared phosphorus determination reagent shall be pale yellow in color. A colorless solution indicates reagent failure, while a blue color suggests phosphorus contamination. The phosphorus determination reagent must be freshly prepared immediately before use, and prepare only the required amount.

(Note: During reagent preparation, prevent phosphorus contamination of double-distilled water, containers and other supplies.)

Storage

The kit has a validity period of 6 months.

Preparation

Spectrophotometer with a wavelength of 660 nm and 1 cm optical path cuvettes (or microplate reader and 96-well plate), 37°C and 45°C water bath or constant temperature incubator, tabletop low-speed centrifuge, pipettes of various specifications, double-distilled water, normal saline (0.9%), vortex mixer, test tubes or centrifuge tubes, beakers or reagent bottles (for preparing phosphorus determination reagent), protein assay reagents (available from our company).

- **Sample handling**

  Accurately weigh the tissue sample. Add 9 times the volume of normal saline at a weight (g): volume (mL) ratio of 1:9. Perform mechanical homogenization under an ice-water bath to prepare 10% tissue homogenate. Centrifuge at 2500 rpm for 10 minutes. Collect the supernatant and further dilute it with normal saline at a ratio of 1:4 to prepare 2% tissue homogenate.

Operation process

1. Enzymatic Reaction:

	Assay tube	Control tube
Reagent 1 (μL)	130	130
Reagent 2 (μL)	80	
Reagent 3 (μL)		120
Reagent 4 (μL)	40	40
Reagent 5 (μL)	40	40
Reagent 6 (μL)	40	
Sample (μL)	100	
Mix well and incubate accurately at 37°C for 10 minutes.		
Reagent 7 (μL)	50	50
Sample (μL)		100
Mix thoroughly, centrifuge at 3500 rpm for 10 minutes, and take 400 μL of the supernatant for phosphorus determination.		

2. Phosphorus Determination Reaction：

	Standard tube	Control tube	Assay tube
0.5 μmol/mL working standard phosphorus solution (μL)	400		
Supernatant of enzymatic reaction (μL)		400	400
Phosphorus Determination Reagent (μL)	2000	2000	2000
Mix thoroughly, incubate in a 45°C water bath for 5 minutes, then cool to room temperature. Set zero with double-distilled water, and measure the absorbance (A) at 660 nm using a 1 cm optical path.			

Note: Due to differences among individual samples, a control tube shall be prepared for each sample. Only 1-2 tubes are required for the standard group and blank group respectively. If the OD

difference between the assay tube and control tube is too small, prolong the first enzymatic reaction time at 37°C for the assay tube (e.g., 20 min or 30 min), to keep the OD difference above 0.01 as far as possible.

3. Simplified Protocol (Recommended for large numbers of samples)

Prior to testing, prepare mixed Solution A and Solution B for control tubes and assay tubes respectively.
Multiply the volume of each reagent specified in the standard operation table by the total number of samples (n) to be tested. Prepare an extra volume equivalent to 1-2 additional tubes to prevent reagent shortage during pipetting. Combine the reagents vertically to obtain Solution A (for control tubes) and Solution B (for test tubes). Refer to the table below for detailed preparation instructions.

	Mixed Solution A	**Mixed Solution B**
Reagent 1 (μL)	130×(n+2)	130×(n+2)
Reagent 2 (μL)		80×(n+2)
Reagent 3 (μL)	120×(n+2)	
Reagent 4 (μL)	40×(n+2)	40×(n+2)
Reagent 5 (μL)	40×(n+2)	40×(n+2)
Reagent 6 (μL)		40×(n+2)
Total volume of mixed reagents (μL)	330×(n+2)	330×(n+2)
Volume to be pipetted per tube (μL)	330	330

① Enzymatic reaction

	Control tube	**Assay tube**
Mixed Solution A (μL)	330	
Mixed Solution B (μL)		330
Sample (μL)		100
Mix well and incubate accurately at 37°C for 10 minutes.		

Reagent 7 (μL)	50	50
Sample (μL)	100	
Mix thoroughly, centrifuge at 3500 rpm for 10 minutes, and take 400 μL of the supernatant for phosphorus determination.		

② Phosphorus Determination Reaction

	Standard tube	**Control tube**	**Assay tube**
0.5 μmol/mL working standard phosphorus solution (μL)	400		
Supernatant of enzymatic reaction (μL)		400	400
Phosphorus Determination Reagent (μL)	2000	2000	2000
Mix thoroughly, incubate in a 45°C water bath for 5 minutes, then cool to room temperature. Set zero with double-distilled water, and measure the absorbance (A) at 660 nm using a 1 cm optical path.			

Calculation

Definition: One enzyme activity unit is defined as the amount of enzyme that decomposes protein to produce 1 μg of amino acid per milligram of tissue protein per minute at 37°C.

Calculation formula:

$$Cu^{2+} - \text{ATPase Activity}(\mu\text{molPi/mgprot/hour}) = \frac{A_{Assay} - A_{Control}}{A_{Standard}} \times C_{Standard} \times N \times \frac{60}{T} \div Cpr$$

Annotation:

$C_{Standard}$: Standard solution concentration, 0.5μmol/mL

T: Reaction time, 10 min

N: Dilution factor of enzymatic reaction system, 4.8

Cpr: Tissue homogenate protein concentration, mg/mL

Precautions

1. It is recommended to use disposable laboratory test tubes for operation.
2. This product is intended for scientific research use only by professionals and must not be used for clinical diagnosis or treatment, in food or drugs, or stored in ordinary residences.
3. This method features microscale detection, high sensitivity and rapid operation. Therefore, the test tubes used must meet strict requirements and be completely phosphorus-free. Disposable phosphorus-removed plastic tubes or centrifuge tubes are recommended. Preventing phosphorus contamination is critical to the success of the assay.
4. Once the phosphorus assay reagent is prepared, do not store it for too long. Its maximum shelf life is one day, and fresh preparation immediately before use is recommended. Keep it refrigerated at all times.
5. All vessels used for reagent preparation must be dedicated, including pipettes for sulfuric acid and containers for water.
6. It is recommended to first prepare the mixed solutions for test tubes and control tubes, then perform the assay following the simplified procedures for higher efficiency and accuracy.