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

H^+/K^+-ATPase Assay Kit

Catalog No.: BC00170

Size: 200T

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	H^+/K^+-ATPase Assay Kit
Detection Method	Colorimetric
Assay Type	Quantitative
Sample Type	Tissue
Detection Instrument	Spectrophotometer (660 nm)

Product Introduction

H^+/K^+-ATPase bears critical physiological significance for gastric acid secretion and gastric digestive function. In the 1970s, Forte et al. first isolated an ATPase from bullfrog gastric mucosa that could be specifically activated by K^+ but not inhibited by ouabain, namely H^+/K^+-ATPase. Later, Lee J et al. verified that membrane vesicles containing this enzyme mediate transmembrane proton transport, laying a molecular foundation for the mechanism of gastric acid secretion. Localized in gastric mucosal parietal cells, H^+/K^+-ATPase belongs to the second class of proton pumps. It accomplishes electroneutral transmembrane H^+/K^+ ion transport via autophosphorylation (E1→E2) and dephosphorylation (E2→E1), continuously pumping protons out of parietal cells to drive gastric acid secretion.

Principle

ATPase catalyzes the hydrolysis of ATP into ADP and inorganic phosphate. The activity of ATPase can be quantified by measuring the yield of inorganic phosphate. H^+/K^+-ATPase is a type of ATPase that is specifically activated by potassium ions and insensitive to inhibition by ouabain.

Components

No.	Components	Size	Notes
Reagent 1	Liquid	10 mL×3	Store at 4°C
Reagent 2	Liquid	7 mL×2	Store at 4°C
Reagent 3	Liquid	8 mL×2	Store at 4°C
Reagent 4	Powder	4 vials	Store at -20°C

Reagent 5	Powder	4 vials	Store at 4°C
Reagent 6	Liquid	3 mL×2	Store at 4°C
Reagent 7	Liquid	10 mL×2	Store at 4°C
Reagent 8	Powder	5 vials	Store at 4°C
Reagent 9	Powder	2 vials	Store at 4°C
Reagent 10	2.5 mol/L sulfuric acid	100 mL×2	Store at RT
Reagent 11	10 mmol/L Phosphorus Standard Stock Solution	10 mL×1	Store at 4°C

Notes:

1. Preparation of **Reagent 4**: For use, dissolve one vial of Reagent 4 powder with double-distilled water to a final volume of 5 mL. Prepare freshly before use; surplus can be stored below -20 °C for up to one week.
2. Preparation of **Reagent 5**: For use, dissolve one vial of Reagent 5 powder with double-distilled water to a final volume of 5 mL. Heat moderately to aid dissolution, and store at 4 °C.
3. Preparation of **Reagent 7**: For use, dilute one vial of liquid Reagent 7 with double-distilled water to a final volume of 25 mL, and store at room temperature.
4. Preparation of **Reagent 8**: For use, dissolve one bottle of Reagent 8 powder with double-distilled water to a final volume of 40 mL. The prepared solution can be stored at 4 °C for one week.
5. Preparation of **Reagent 9**: For use, dissolve one bottle of Reagent 9 powder with double-distilled water to a final volume of 100 mL, and store at room temperature.
6. Preparation of 0.5 μmol/mL phosphorus standard solution: Mix the 10 mmol/L phosphorus standard stock solution and double-distilled water at a volume ratio of 1:19. For example, take 0.5 mL stock solution and add 9.5 mL double-distilled water.
7. Preparation of Phosphorus Determination Reagent: Mix double-distilled water, 2.5 mol/L sulfuric acid, Reagent 8 and Reagent 9 at a volume ratio of 2:1:1:1. The properly prepared reagent shall be pale yellow. A colorless solution indicates invalid reagents, while a blue color suggests phosphorus contamination. Prepare the phosphorus color-developing reagent immediately prior to use.

Storage

The kit has a validity period of 6 months.

Preparation

Visible spectrophotometer equipped with 660 nm wavelength and 1 cm path length cuvettes (or microplate reader), 37-45 °C water bath or incubator, low-speed tabletop centrifuge, adjustable pipettes of various volumes, double-distilled water, 0.9% normal saline, vortex mixer, test tubes or centrifuge tubes, protein quantification reagent (for tissue and cell samples, available from our company).

Sample handling

Accurately weigh the gastric mucosal tissue. Add 9 volumes of normal saline at a mass (g) to volume (mL) ratio of 1:9, and prepare 10% tissue homogenate under ice-water bath conditions. Centrifuge the homogenate at 2500 rpm for 10 min, collect the supernatant, and dilute it 5-fold with normal saline to a concentration of 2% for subsequent detection.

Operation process

1. Enzymatic reaction:

	Control tube	Assay 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
Samples (μL)		100
Mix thoroughly and incubate in a 37 °C water bath for 10 minutes.		
Reagent 7 (μL)	50	50
Samples (μL)	100	
Mix thoroughly, centrifuge at 3500 rpm for 10 min, collect 400 μL of supernatant. The supernatant is used 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.			

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:

$$H^+/K^+ - \text{ATPase Activity(U/mg)} = \frac{A_{Assay} - A_{Control}}{A_{Standard}} \times C_{Standard} \times N \div \frac{T}{60} \div Cpr$$

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.