Hydrogen Potassium ATPase Activity Assay Kit

(Cat/No.:BC125 Size:100T/48S,200T/96S)

1. Principle of Measurement

ATPase catalyzes the hydrolysis of ATP and one of the product phosphate can be determined to measure the activity of ATPase. H^+K^+-ATPase is activated specially by potassium ion and cannot be inhibited by Ouabain like other types of ATPase.

2. Compositions and Preparation

	State	200T/96 Samples	100T/48 Samples	Storage Temperature
Reagent I	Liquid	3 Bottles×10ml	2 Bottles×10ml	4°C
Reagent II	Liquid	2 Bottles×7ml	1 Bottle×7ml	4°C
Reagent II	Liquid	2 Bottles×8ml	1 Bottle×8ml	4°C
Reagent IV	Powder	4 Bottles	2 Bottles	-20°C
Preparation of Reagent IV Solution：Dissolve one bottle of powder with double distilled water (DDW) to a final volume of 5ml prior to use and the solution is stable for a week under -20°C.				
Reagent V	Powder	4 Bottles	2 Bottles	4°C
Preparation of Reagent V Solution：Dissolve one bottle of powder with DDW to 5 ml and the mixture can be warmed to accelarate the dissolution. Solution should be preserved at 4°C.				
Reagent VI	Liquid	2 Bottles×3ml	1 Bottle×3ml	4°C
Reagent VII	Liquid	2 Bottles×10ml	1 Bottle×10ml	4°C
Prepartion of Reagent VII Solution：Disperse reagent VII with DDW to 25ml and stored at RT.				
Reagent VIII	Powder	5 Bottles	3 Bottles	4°C
Preparation of Reagent VIII Solution :Dissolve each bottle of powder with DDW to 40ml and the solution is stable within a week at 4°C.				
Reagent IX	Powder	2 Bottles	1 Bottle	4°C
Preparation of Reagent IX Solution：				
Reagent X	Liquid	2 Bottles×100ml	1 Bottle×100ml	RT
Reagent XI	Liquid	1 Bottle×10ml	1 Bottle×10ml	4°C
Preparation of Reagent XI Standard Solution (0.5μmol/mL Phosphate Standard Solution): Extract 0.5ml Reagent XI and dilute with DDW to a final volume of 10ml.				
Preparation of Phosphate Determinant：Mix DDW, Reagent X, Reagent VIII and IX solution with the ratio 2 :1 :1 :1 (v/v). And the determinatant appears to be light yellow. The reagent with no effciency is transparent and the contaminated reagent is blue. The reagent should be prepared prior to use.				

3. Sample Pre-Treatment

Weigh gastric mucosa tissues and add physiological saline with the ratio 1 :9 (w/v). Homogenize and centrifuge at 2,500 rpm for 10 min. Dilute the supernatant with saline to five times the initial supernatant volume and set aside for measurement.

4. Standard Procedures of Measurement

I. Enzyme Catalyzed Reaction

Compositions (μl)	Control	Sample
Reagent I	130	130
Reagent II		80
Reagent III	120	
Reagent IV	40	40
Reagent V	40	40
Reagent VI		40
Sample		100
Mixed and warmed at 37°C for 10 min		
Reagent VII	50	50
Sample	100	

Mix and centrifuge at 3,500 rpm for 10 min and extract 400μl supernatant from each tube.

II. Phosphate Determination

Compositions (μl)	Standard	Control	Sample
0.5μmol/mL Standard Phosphate	400		
Supernatant from Rference tube		400	
Supernatant from Sample tube			400
Phosphate Determinant	2000	2000	2000

Mix and warm at 45°C for 5 min. Cool to room temperature. Regulate the spectrophotometer with DDW and record the OD values of each tube at 660 nm with 1cm path length.

5. Simple Method for Mass No. Of Samples

I. Preparation of Sample Solution and Reference Solution for Assay Purpose

The amount of each reagent added is based on the total number of samples n with additional amount to avoid the shortage of mixture. Solution A is prepared for reference purpose while solution B is prepared for sample measurement purpose.

Compositons (μl)	Solution A	Solution B
Reagent I	130×(n+2)	130×(n+2)
Reagent II		80×(n+2)
Reagent III	120×(n+2)	
Reagent IV	40×(n+2)	40×(n+2)
Reagent V	40×(n+2)	40×(n+2)
Reagent VI		40×(n+2)
Total Volume	330×(n+2)	330×(n+2)
Addition Amount for Each Sample	330	330

II. Measurement Procedures

a. Enzyme Catalyzed Reaction

Compositions (μl)	Reference	Sample
Solution A	330	
Solution B		330
Sample		100
Mix and warm at 37°C for 10 min		
Reagent VII	50	50
Sample	100	

Mix and centrifuge at 3,500 rpm for 10 min and extract 400μl supernatant from each tube.

b. Phosphate Determination

Exactly the same as the phosphate determination step mentioned above.

6. Calculation Formula and Example

I. Definition

One ATPase activity unit is defined as one μmol phosphate generated by the hydrolysis of ATP catalyzed by ATPase from 1mg protein within an hour.

II. Formula

$$\underset{\text{U/mg}}{H^+k^+\text{ATpase Activity}} = \frac{OD_{sample} - OD_{Ref}}{OD_{standard}} \times \underset{0.5\mu\text{mol/ml}}{c_{standard}} \times \frac{coD}{4.8} \div \frac{\text{Time}}{\frac{1}{6}\text{ h}} \div \underset{\text{mg/ml}}{c_{prot}}$$

Note : CoD represents for coefficient of dilution.

III. Example

2% rat gastric mucosa tissue homogenate was prepared and measured. The OD values were 0.769, 0.189 and 0.689 respectively. The protein concentration of the homogenate was measured to be 1.159mg/ml.

$$H^+K^+ATPase\ Activity\ U/mg = \frac{OD_{Sample} - OD_{Ref}}{OD_{Standard}} \times \frac{C_{standard}}{0.5\mu mol/ml} \times \frac{CoD}{4.8} \div \frac{Time}{\frac{1}{6}h} \div \frac{C_{prot}}{mg/ml}$$

$$= \frac{0.689 - 0.189}{0.769} \times \frac{0.5\mu mol}{ml} \times 4.8 \div \frac{1}{6}h \div \frac{1.159mg}{ml} = 8.08U/mg$$

7. Note

I. This method is fast and is available for low enzymatic activity determination. However, the test tubes used for this method are required to be clean and no phosphate is allowed in the test tube. Please clean the tube for the measurement carefully after containing phosphate solution by boiling the water in the tube with detergent and washing with distilled water. Also, the usage of disposable plastic tubes or new glass tubes is recommended. To avoid the contamination is the key to a successful measurement.

II. The phosphate determination agent is unstable and should be prepared prior to the measurement.

III. It is recommended to adopt the simple method which would quicken the measurement with more reliable results.

IV. The vessels should be specific for containment of different reagents including the water container or the pipette for sulfuric acid.

8. Significance of Measurement

Hydrogen potassium ATPase is the enzyme primarily responsible for the acidification of the stomach contents and the activation of the digestive enzyme pepsin. It was first discovered by Forte et al in the 1970s as a kind of enzyme separated from bullfrog's gastric mucosa and can be activated specifically by potassium ion but cannot be inhibited by Ouabain. Afterward, it was proven that the membrane vesicles containing this enzyme functions for transmembrane transport which would lay the molecular foundation for the mechanism of gastric acid secretion. The H+/K+ ATPase is found in parietal cells, which are highly specialized epithelial cells located in the gastric mucosa. As a type of P2-ATPase, H+/K+ ATPase transports one proton into lumen for each potassium ion retrieved from lumen with the energy from hydrolysis of ATP. This electroneutral transport extrudes protons as the final step of gastric acid secretion.