Ca2+Mg2+-ATPase assay kit (Tissue)

(Cat.No.:BC111 Size:100T/48S,50T/25S)

1. Significance

ATPase exists in tissue cells or on the cell membranes. As a type of protease anchored on the cell membrane, it plays an important role in transmission of energy or information and transport of substance. Under the circumstance of hypoxia or suffering certain diseases, the activity of ATPase may vary from the ordinary activity. Also, genetic disorder may cause the activity out of ordinary.

2. Principle of Measurement

ATPase catalyzes the hydrolysis of ATP with the product of ADP and phosphate. The amount of phosphate can be determined and thus measure the activity of ATPase.

3. Composition and Preparation (The kit is valid for 6 months)

	State	50T/24 Samples	100T/ 48 Samples	Preservation
Reagent I	Liquid	1 Bottle×13ml	2 Bottles×13ml	4°C for 6 months
Reagent II	Liquid	1 Bottle×4ml	2 Bottles×4ml	4°C for 6 months
Reagent III	Powder	4 Bottles	8 Bottles	-20°C for 6 months
Preparation of Reagent III: Dissolve each bottle of powder with 1ml double distilled water (DDW) and the excess can be preserved at -20°C for a week.				
Reagent IV	Liquid	1 Bottle×5ml	2 Bottles×5ml	4°C for 6 months
Reagent V	Solution A	4 Bottles×7ml	8 Bottle×7ml	4°C for 6 months
	Solution B	4 Bottles×6ml	8 Bottles×6ml	4°C for 6 months (avoid light)
Note: Gel may generated at low temperature and under this circumstance, solution should be warmed at 60°C for 10 min to fully dissolve. Both Solution should be kept away from phosphate contamination.				
Reagent VI	Liquid	1 Bottle×50ml	2 Bottles×50ml	RT for 6 months
Reagent VII	Liquid	1 Bottle×5ml	1 Bottle×5ml	4°C for 6 months
Reagent VIII	Liquid	1 Bottle×4ml	2 Bottles×4ml	4°C for 6 months
Reagent IX	Powder	4 Bottles	8 Bottles	4°C for 6 months
	Diluent	4 Bottles×0.5ml	8 Bottles×0.5ml	4°C for 6 months
Preparation of Reagent IX: Dilute 1 bottle of powder with 1 bottle of diluent and the excess can be preserved at 4°C				
DDW		1 Bottle×40ml	1 Bottle×40ml	4°C for 6 months
Preparation of 0.1mM Phosphate Solution: Dilute 0.1ml reagent VII with DDW to a final volume of 10ml.				
Preparation of 0.02mM Standard Phosphate Solution: Dilute 0.1mM solution (1ml) with DDW to 5ml.				
Preparation of Substrate Solution: Mix reagent I, II and III at the ratio of 13:4:4 (v/v) prior to use.				
Preparation of Reagent V (Chromogenic Agent): 0.5 h prior to use, pre-warm solution B. Reagent V (A) is then added and fully mix the solution. The solution can be preserved at 2-8°C for 5 days and is capable of measurement of 13 tubes. Also, the solution A and B can be mixed with the ratio 7:6 (v/v) if small amount of reagent V needed.				

4. Sample Pre-Treatment

I. Tissues

Weigh the tissues precisely and add physiological saline with the ratio 1:9 (g/ ml). Homogenize in an ice water bath and centrifuge the homogenate at 2,500 rpm for 10 min. Extract the supernatant and dilute with saline with the ratio 1:9 (v/v) and 1% tissue homogenate is formed. The protein concentration can be measured with Coomassie Brilliant Blue method. Were the protein concentration to high, dilute the 1% homogenate to the desired concentration.

II. Cultured Cells

Digest the cultured cells and centrifuge the solution. Discard the supernatant and add saline so that the final concentration of cells is 10^7/ml. There are three methods for cell disruption.

a. Homogenize the mixture.

b. Sonicate the mixture.

c. Freeze and thaw the mixture for 3 times. (This may lower the activity).

The protein concentration can be measured with Coomassie Brilliant Blue method. Were the protein concentration to high, dilute the homogenate to the desired concentration.

Note: Shake to mix the sample before the addition.

Note: Samples cannot be diluted with phosphate in presence.

Note: Pre-experiment should be done in order to let the absolute absorbance (Sample absorbance subtracting Reference absorbance) to be around 0.2.

5. Standard Procedures

I . Enzymatic Reaction

Compositions (ml)	Reference	Sample
DDW	0.16	
Sample		0.1
Reagent VIII		0.08
Reagent IX		0.08
Reagent I	0.26	0.26
Reagent II	0.08	0.08
Reagent III	0.08	0.08
Mix and Warm at 37°C for 10 min		
Reagent IV	0.1	0.1
Sample	0.1	

Mix and centrifuge at 3,500 rpm for 10 min and extract supernatant.

II . Phosphate Determination

Compositions (ml)	Blank	Standard	Reference	Sample
DDW	0.3			
0.02mM Standard		0.3		

Phosphate Solution				
Supernatant			0.3	0.3
Reagent V	1	1	1	1
Mix and Set Aside for 2 min at RT				
Reagent VI	1	1	1	1

Mix and set aside for 5 min at RT. Regulate the spectrophotometer at 636nm with DDW. Record the absorbed optical density (OD) for each tube with 1cm light path.

6. Simple Method

I. Enzymatic Reaction

Composition (ml)	Control	Sample
DDW	0.16	
Sample		0.1
Reagent VIII		0.08
Reagent IX		0.08
Substrate Solution	0.42	0.42
Mix and Warm at 37°C for 10 min		
Reagent IV	0.1	0.1
Sample	0.1	

Mix and centrifuge at 3k-4k rpm for 10 min and extract supernatant for phosphate determination.

Note: The preparation of substrate solution is mentioned at page 1.

II. Phosphate Determination

Exactly the same as the phosphate determination step mentioned above.

	Blank well	Standard well	Control well	Sample well
DDW（mL）	0.3			
0.02μmol/mL Phosphorus standard solution（mL）		0.3		
Supernatant（mL）			0.3	0.3
Reagent 5, colorimetric reagent（mL）	1.0	1.0	1.0	1.0
Mix well and let stand at room temperature for 2 minutes.				
Reagent 6（mL）	1.0	1.0	1.0	1.0
Mix well, let stand at 37℃ for 5-10 minutes, and measure the absorbance of each tube at 636nm with a 1cm optical path using double-distilled water as the zero point.				

Note: The cuvettes should be washed with tap water 10 times and then washed with DDW for 4-5 times to avoid contamination by phosphate.

7. Calculation Formula and Example

I. Tissue Samples

a. Definition

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

b. Formula

ATpase Activity U/mg

$$= \frac{0.360 - 0.280}{0.255 - 0.052} \times 0.02 \div \frac{1}{6} \times 7.8 \div (0.998 \div 10) = 8.316_{\mathrm{u/mg}}$$

$$\underset{U/mg}{ATPase\ Activity} = \frac{OD_{Sample} - OD_{Ref}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{0.02mM} \div \frac{Time}{\frac{1}{6}h} \times \frac{CoD}{7.8} \div \frac{C_{Protein}}{mg/ml}$$

8. Measurement Significance

ATPase is a protease found on the membranes of tissue cells and organelles, playing a crucial role in substance transport, energy conversion, and information transmission. The activity of this enzyme undergoes a series of changes under conditions such as h ypoxia and certain diseases, and it is also associated with some genetic disorders. 10. Measurement Principle: ATPase can decompose ATP to produce ADP and inorganic p hosphate. Measuring the amount of inorganic phosphate can determine the activity level of ATPase.

9. Measurement Principle

ATPase can break down ATP to produce ADP and inorganic phosphorus. The amount of inorganic phosphorus can be measured to determine the level of ATPase activity.