ATP Assay Kit

(Cat/No.:BC102 Size:100T/48S)

1. Significance of Measurement

Adenosine triphosphate (ATP) is the basic carrier for energy transfer in vivo. Thus the concentration variation will infect the energy metabolism within organs. ATP as the most important energy molecule plays an important role in the physiology and pathology process of cells. The variation of ATP concentration would affect the functions of cells and the concentration would decrease for cells undergoing apoptosis or necrosis process and for cells in toxic conditions. Stimulation of cells by high glucose concentration may increase the ATP level in cells. Normally, the decrease of ATP level may be caused by the impaired or decreased functions of mitochondria and is often accomplished with the decrease of mitochondrial transmembrane potential in apoptosis process. This assay kit is designed for the the ATP level determination of red blood cells or tissues.

2. Principle of Measurement

Creatine and ATP is catalyzed by creatine kinase to give phosphocreatine. The product can further react with chromogenic reagent with the colored compound generated. Thus the ATP concentration can be calculated based on it.

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

	Composition	Size	Preservation Temperature
Reagent I	Substrate I	1 Bottle of Powder	RT
Preparation of Reagent I Solution: Dissolve the bottle of powder with boiled double distilled water (DDW) to 10 ml final volume with powder fully dissolved. Were crystals observed in the solution prior to use, boil the solution and place the mixture at 37°C.			
Reagent II	Substrate II	1 Bottle×20ml	4°C
Reagent III	Catalyzer	2 Bottles of Powder	-20°C
		2 Bottle×760µl	4°C
		Before use, add one vial of Reagent 3 powder to one vial of Reagent 3 liquid and dissolve it before use.	
Reagent IV	Precipitant	1 Bottle×5.5ml	4°C
Reagent V	Chromogenic Agent	4 Bottles×7ml (A)	4°C
		4 Bottles×6ml (B)	4°C
Preparation of Reagent V Solution: Mix solution A and solution B with the ratio 7:6 (v/v) prior to use with the amount needed. The reagent V should be preserved at 4°C.			
Reagent VI	Terminator	1 Bottle×50ml	RT
Reagent VII	ATP Stock Solution	2 Bottles of Powder	4°C
5mM ATP Solution Preparation: Dissolve one bottle of powder with DDW to 1ml final volume.			
Preparation of Reagent VII Solution (1mM ATP Solution): Dilute the 5mM solution with DDW to 5 times its initial volume.			

Note: The validity of the sealed kit is 3 months and is 1 month after unsealing.

4. Pretreatment

1. Red Blood Cells

Extract the packed RBCs in whole blood samples with anticoagulant treated and add DDW with the ratio 1 ml RBCS to 4 ml DDW. Mix thoroughly and the resulted hemolytic solution should be transferred to glass tube and boiled for 10 min. Swirl the boiled mixture for 1 min and centrifuge at 4,000 rpm for 10 min. Extract the supernatant for measurement.

2. Tissue Samples

Weigh the tissue precisely and for every 1 g tissues, add 9 ml boiled DDW and homogenize the mixture. The homogenate should be then boiled for 10 min and put on vortex for 1 min. Centrifuge the boiled homogenate at 3,500 rpm for 10 min. Extract the supernatant for measurement.

3. Cultured Cells

Centrifuge the sample to separate the cells and culture medium. Discard the medium and add 0.3-0.5 ml hot DDW to the pellets. Warm the mixture in a water bath at 90-100°C and homogenize to disrupt the cells. Then boiled the disrupted cells for 10 min and put on vortex for 1 min. The solution can be measured directly.

5. Standard Method Procedures

Compositions (μl)	Blank	Standard	Sample	Comparison
Reagent VII Solution	30	30		
Sample			30	30
Reagent I	100	100	100	100
Reagent II	200	200	200	200
Reagent III		30	30	
DDW	30			30
Mix thoroughly and warm at 37° C for 30 min.				
Reagent IV	50	50	50	50
Mix thoroughly and centrifuge at 4,000 rpm for 5 min. Extract the supernatant.				
Supernatant	300	300	300	300
Reagent V	500	500	500	500
Mix and set aside at RT for 2 min.				
Reagent VI	500	500	500	500

Mix and set aside at RT for 5 min. Zero the 0.5cm path length-cuvettes at 636 nm with DDW and record the optical density (OD) value of each tube.

Note: the cuvettes should be washed 10 times with tap water and 4-5 times with DDW before the measurement in order to avoid phosphate contamination.

6. Simple Method Procedures

1. Preparation of Working Fluid

   Working Fluid A: Mix reagent I, II and III with the ratio 10:20:3 prior to use with the desired amount.

   Working Fluid B: Mix reagent I, II with the ratio 1:2 prior to use with the desired amount.

2. Procedures

Compositions (μl)	Blank	Standard	Sample	Comparison
Reagent VII Solution	30	30		
Sample			30	30
Working Fluid A		330	330	
Working Fluid B	300			300
DDW	30			30
Mix thoroughly and warm at 37° C for 30 min.				
Reagent IV	50	50	50	50
Mix thoroughly and centrifuge at 4,000 rpm for 5 min. Extract the supernatant.				
Supernatant	300	300	300	300
Reagent V	500	500	500	500
Mix and set aside at RT for 2 min.				
Reagent VI	500	500	500	500

Mix and set aside at RT for 5 min. Zero the 0.5cm path length-cuvettes at 636 nm with DDW and record the optical density (OD) value of each tube.

Note: the cuvettes should be washed 10 times with tap water and 4-5 times with DDW before the measurement in order to avoid phosphate contamination.

7. Calculation Formula and Examples

1. Formula

i. For Red Blood Cells.

$$\frac{ATP\ Conc.}{\mu mol/g} = \frac{OD_{Sample} - OD_{Comparison}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1{,}000\mu M} \times CoD \div \frac{C_{Hemoglobin}}{g/L}$$

ii. For Tissues and Cultured Cells

$$\frac{ATP\ Conc.}{\mu mol/g} = \frac{OD_{Sample} - OD_{Comparison}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1{,}000\mu M} \times CoD \div \frac{C_{protein}}{g/L}$$

Note: CoD represents coefficient of dilution.

2. Examples

i. 10% intestine homogenate was prepared and diluted with DDW to 4 times of its initial volume. The OD values were 0.070, 0.443, 0.728 and 0.660 respectively. The protein concentration was 0.4970g/L.

$$\underset{\mu mol/g}{ATP\ Conc.} = \frac{OD_{Sample} - OD_{Comparison}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1{,}000\mu M} \times CoD \div \frac{C_{protein}}{g/L}$$
$$= \frac{0.728 - 0.660}{0.443 - 0.070} \times 1000 \times 4 \div 0.4970 = 1.467 \times 10^3 \mu mol/g$$

ii. Prepared cell suspension was measured with OD values equal to 0.071, 0.442, 0.507 and 0.481 respectively. The protein concentration was 1.178 g/L.

$$\underset{\mu mol/g}{ATP\ Conc.} = \frac{OD_{Sample} - OD_{Comparison}}{OD_{Standard} - OD_{Blank}} \times \frac{C_{Standard}}{1{,}000\mu M} \times CoD \div \frac{C_{protein}}{g/L}$$
$$= \frac{0.507 - 0.481}{0.442 - 0.071} \times 1000 \times 1 \div 1.178 = 5.949 \times 10^1 \mu mol/g$$

8. Notes

1. This method is fast and sensitive for trace amount of sample. However, the test tube should be clean as the prevention of phosphate contamination is the key to success. It is recommended use the disposable plastic tube to avoid phosphate contamination.
2. The disposable plastic tubes are available in our shop and it is recommended to purchase the tubes along with the kit.
3. Chromogenic agent is recommended to prepare prior to use. The agent is stable within 5 days in refrigerator.
4. Hemoglobin concentration can be measured by the hemoglobin assay kit (C021) from the institute. The protein concentration of tissues can be measured by bicinchoninic acid assay kit (A045-3) or protein assay kit (Coomassie Brilliant Blue, A045-2) available in the institute.
5. The optimum tissue homogenate concentration is around 2-5% and were the concentration too high with high OD values, it is recommended to do the pre-experiment and dilute the homogenate so that the OD values of comparison tubes are below 1.0.