Technical Manual

ATP Colorimetric Assay Kit

- **Catalogue Code: MAES0119**
- **Size: 100Assays**
- **Research Use Only**

1. Key Features and Sample Types:

Detection method:

Colorimetric method

Specification:

100Assays

Range:

0.01-1.5 mmol/L

Sensitivity:

0.01 mmol/L

Storage:

2-8°C and -20°C for 6 months

Expiry:

See Kit Label

Experiment Notes:

This kit is for **research use only.**

Instructions should be strictly followed. Changes of operation may result in unreliable results.

The validity of kit is 6 months.

Do not use components from different batches of kit.

2. Background:

Adenosine-5'-triphosphate (ATP) is a natural nucleotide present in every cell, an organic compound composed of purine base (adenine), ribose and 3 phosphate groups. The content of ATP in tissue or cells is generally in a dynamic balance state, which is of great significance to constitute a stable energy supply environment inside the organism. The release of ATP from many cells is a physiological or pathophysiological response to mechanical stress, hypoxia, inflammation and some agonists.

3. Intended Use:

This kit can be used to measure ATP content in tissue samples.

4. Detection Principle:

Creatine Kinase catalyzes adenosine triphosphate and creatine to produce creatine phosphate, then detected by phosphomolybdic acid colorimetry.

5. Kit Components & Storage:

Item	Specification	Storage
Extracting Solution	60 mL × 1 vial	2-8°C, 6 months
Substrate	Lyophilized × 2 vials	2-8°C, 6 months
Buffer Solution	24 mL × 1 vial	2-8°C, 6months
Enzyme Reagent	Lyophilized × 2 vials	-20°C, 6 months
Protein Precipitator	6 mL × 1 vial	2-8°C, 6 months
Chromogenic Agent A	48 mL × 1 vial	2-8°C, 6 months, avoid direct sunlight
Chromogenic Agent B	16 mL × 1 vial	2-8°C, 6 months
Stop Solution	60 mL× 1 vial	2-8°C, 6 months
Standard	Lyophilized × 4 vials	2-8°C, 6 months

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Spectrophotometer (636 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water
- Normal Saline (0.9% NaCl)

6. Assay Notes:

1. Fresh samples should be used.

2. Preventing phosphorous pollution is key for this assay, it is recommended to use disposable test tubes.

7. Reagent Preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate application solution:** Dissolve a vial of substrate fully with 6 mL of boiled double distilled water. If the prepared solution appear crystal before assay, incubate in boiling water bath to dissolve fully and then store at 37°C for assay. The prepared solution can be stored at 2-8°C for 7 days.

3. Preparation of **enzyme application solution:** Dissolve a vial of enzyme reagent fully with 1.8 mL of double distilled water. The prepared solution can be stored at -20°C for 7 days.

4. Preparation of **control working solution:** Mix the substrate application solution, buffer solution, double distilled water at the ratio of 100:200:30 fully. Prepare the needed amount fresh solution before use.

5. Preparation of **detection working solution:** Mix the substrate application solution, buffer solution, enzyme application solution at the ratio of 100:200:30 fully. Prepare the needed amount fresh solution before use.

6. Preparation of **chromogenic agent:** Mix the chromogenic agent A and chromogenic agent B at the ratio of 3:1 fully. Place it at 37°C for 1 hour. Prepare the needed solution before use.

7. Preparation of **ATP standard stock solution (10 mmol/L):** Dissolve a vial of standard with 1 mL of double distilled water fully. The prepared solution can be stored at -20°C for 7 days.

8. Preparation of **ATP standard solution (1 mmol/L):** Dilute 10 mmol/L ATP standard stock solution with double distilled water for 10 times. The prepared solution can be stored at -20°C for 7 days.

8. Sample Preparation:

Tissue sample:

Weigh the tissue accurately, cut into pieces, then adding 9 times of the volume of extracting solution according to the ratio of weight (g): volume (mL) =1:9. Homogenize tissue with homogenizer instrument (60 Hz, 90s) in the ice bath. Then incubate in boiling water bath for 2 min, and cool the tubes to room temperature with running water. Centrifuge at 10 000 g for 10 min, then take the supernatant for detection.

Sample Notes:

The concentration should be determined before preforming the assay. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

If the sample type is not included in the manual, a preliminary experiment is suggested to verify the validity.

Dilution of Samples:

Large variances in results may be seen when performing pre-experiments. Dilute the sample according to the result of the pre-experiment and the detection range (0.01-1.5 mmol/L).

The recommended dilution factor for different samples is as follows (for reference only).

Sample Type:	Dilution Factor:
10% Rat muscle tissue homogenate	2-4
10% Rat liver tissue homogenate	2-4
10% Mouse brain tissue homogenate	2-4
10% Rat kidney tissue homogenate	2-4
10% Rat lung tissue homogenate	2-4

Note: The diluent is double distilled water.

9. Assay Protocol:

Ambient Temperature: 25-30°C

Optimum detection wavelength: 636 nm

10. Operation Steps:

1. **Blank tube:** Take 30 μL of ATP standard solution (1 mmol/L) to the 1.5 mL EP tube, then add 330 μL of control working solution.
   Standard tube: Take 30 μL of ATP standard solution (1 mmol/L) to the 1.5 mL EP tube, then add 330 μL of detection working solution.
   Control tube: Take 30 μL of sample supernatant to the 1.5 mL EP tube, then add 330 μL of control working solution.
   Sample tube: Take 30 μL of sample supernatant to the 1.5 mL EP tube, then add 330 μL of detection working solution.

2. Mix fully and incubate at 37°C for 30 min.

3. Add 50 μL of protein precipitator to each tube.

4. Mix fully for 3 s and centrifuge at 10000 g for 5 min, then take 300 μL of supernatant to measure according to the following steps.

5. Add 500 μL of chromogenic agent to each tube.

6. Mix fully and stand for 2 min at room temperature.

7. Add 500 μL of stop solution to each well.

8. Mix fully and stand for 5 min at room temperature. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 636 nm wavelength with 0.5 cm optical path cuvette.

Operation Table

	Blank tube	Standard tube	Control tube	Sample tube
ATP standard solution (1 mmol/L) (μL)	30	30		
Sample supernatant (μL)			30	30
Control working solution (μL)	330		330	
Detection working solution (μL)		330		330
Mix fully and incubate at 37°C for 30 min.				
Protein precipitator (μL)	50	50	50	50
Mix fully for 3s and centrifuge at 10000 g for 5 min, then take 300 μL of supernatant to measure according to the following steps.				
Supernatant (μL)	300	300	300	300
Chromogenic agent (μL)	500	500	500	500
Mix fully and stand for 2 min at room temperature				
Stop solution (μL)	500	500	500	500
Mix fully and stand for 5 min at room temperature. Set the spectrophotometer to zero with double distilled water and measure the OD values of each tube at 636 nm wavelength with 0.5 cm optical path cuvette.				

11. Calculations:

Tissue sample:

$$\text{ATP content (mmol/kg wet weight)} = \frac{OD_{Sample}-OD_{Control}}{OD_{Standard}-OD_{Blank}} \times c \div \frac{m}{V_1} \times f$$

c: The concentration of standard, 1 mmol/L

f: Dilution factor of sample before tested

m: The weight of tissue sample, g

V_1: The volume of extracting solution in the sample pretreatment step of tissue sample, mL

12. Performance Characteristics:

Detection Range	0.01-1.5 mmol/L
Sensitivity	0.01 mmol/L
Average recovery rate (%)	103
Average inter-assay CV (%)	9.3
Average intra-assay CV (%)	3.6

Analysis

For crucian muscle tissue, dilute with double distilled water for 3 times, carry the assay according to the operation table. The results are as follow.

The results are as follows:

The average OD value of the blank is 0.048, the average OD value of the standard is 0.622, the average OD value of the sample is 0.761, the average OD value of the control is 0.758, and the calculation result is:

$$\textbf{ATP (mmol/kg fresh weight)} = \frac{0.761-0.758}{0.622-0.048} \times 1 \div 0.1 \times 0.9 \times 3$$

$$= 0.14 \text{ mmol/kg fresh weight}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Prevent touching skin and clothing.

Wash immediately with plenty of water if touching it carelessly.

All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

Before the experiment, read the instructions carefully, and wear gloves and work clothes

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