Technical Manual

ATP Colorimetric Assay Kit

- **Catalogue Code: MAES0118**
- **Size: 96T**
- **Research Use Only**

1. Key Features and Sample Types

Detection method:

Colorimetric method

Specification:

96T

Range:

0.03-1.5 mmol/L

Sensitivity:

0.01 mmol/L

Storage:

2-8°C and -20°C for 12 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 12 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 animal tissue sample.

4. Detection Principle

Creatine kinase catalyzes adenosine triphosphate and creatine to produce creatine phosphate. The content of phosphocreatine was determined by colorimetric method to reflect the content of ATP.

5. Kit Components & Storage

Item	Specification	Storage
Extracting Solution	60 mL × 1 vial	2-8°C, 12 months
Substrate	Lyophilized × 2 vials	2-8°C, 12 months
Buffer Solution	24 mL × 1 vial	2-8°C, 12 months
Enzyme Reagent	Lyophilized × 2 vials	-20°C, 12 months
Protein Precipitator	6 mL × 1 vial	2-8°C, 12 months
Chromogenic Agent A	12 mL × 1 vial	2-8°C, 12 months, Avoid direct sunlight
Chromogenic Agent B	4 mL × 1 vial	2-8°C, 12 months
Stop Solution	12 mL× 1 vial	2-8°C, 12 months
Standard	Lyophilized × 4 vials	2-8°C, 12 months
Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Incubator
- Centrifuge
- Microplate Reader (630-640 nm)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

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.

3. When the OD value is more than 1, it is necessary to increase the dilution ratio and detect again

7. Reagent preparation:

1. Bring all reagents to room temperature before use.

2. Preparation of **substrate application solution:** Dissolve a vial of substrate with 6 mL of boiled double distilled water. If the prepared solution is crystallised before assay, incubate in boiling water bath to dissolve and then store at 37°C. 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 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. Prepare 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. Prepare fresh solution before use.

6. Preparation of **chromogenic agent:** Mix the chromogenic agent A and chromogenic agent B at the ratio of 3:1. Place it at 37°C for 1 hour. Prepare 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. 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 10 times. The prepared solution can be stored at -20°C for 7 days.

9. Sample Preparation

Tissue sample:

Weigh the tissue accurately, cut into pieces, then add 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 10000 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 (0.03-1.5 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.

10. Assay Protocol

Ambient Temperature: 25-30°C

Optimum detection wavelength: 636 nm

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S7'	S7	S15'	S15	S23'	S23	S31'	S31	S39'	S39
B	B	B	S8'	S8	S16'	S16	S24'	S24	S32'	S32	S40'	S40
C	S1'	S1	S9'	S9	S17'	S17	S25'	S25	S33'	S33	S41'	S41
D	S2'	S2	S10'	S10	S18'	S18	S26'	S26	S34'	S34	S42'	S42
E	S3'	S3	S11'	S11	S19'	S19	S27'	S27	S35'	S35	S43'	S43
F	S4'	S4	S12'	S12	S20'	S20	S28'	S28	S36'	S36	S44'	S44
G	S5'	S5	S13'	S13	S21'	S21	S29'	S29	S37'	S37	S45'	S45
H	S6'	S6	S14'	S14	S22'	S22	S30'	S30	S38'	S38	S46'	S46

Note: A, blank wells; B, standard wells; S1'-S46', control wells; S1-S46, sample wells.

10. Operation Steps

Enzymatic reaction

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.

2. **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.

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

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

5. Mix fully for 3s and centrifuge at 10000 g for 5 min, then take supernatant of each tube for detection.

Color reaction

1. Take 60 μL of supernatant to corresponding wells.
2. Add 100 μL of chromogenic agent to each well.
3. Mix fully for 5s with microplate reader and stand for 2 min at room temperature.
4. Add 100 μL of stop solution to each well.
5. Mix fully for 5s with microplate reader, stand at room temperature for 5 min, and measure the OD value of each well at 636 nm.

Operation Table

Enzymatic reaction

	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 3 s and centrifuge at 10000 g for 5 min, then take supernatant for detection.				

Color reaction

	Blank well	Standard well	Control well	Sample well
Supernatant (μL)	60	60	60	60
Chromogenic agent (μL)	100	100	100	100
Mix fully and stand for 2 min at room temperature				
Stop solution (μL)	100	100	100	100
Mix fully for 5s with microplate reader, stand at room temperature for 5 min, and measure the OD value of each well at 636 nm.				

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	0.03-1.5 mmol/L
Sensitivity	0.01 mmol/L
Average recovery rate (%)	94
Average inter-assay CV (%)	5.4
Average intra-assay CV (%)	5.1

Analysis

For crucian muscle tissue, dilute with double distilled water for 3 times, carryout the assay according to the operation table.

The results are as follows:

The average OD value of the blank is 0.104, the average OD value of the standard is 0.526, the average OD value of the sample is 0.931, the average OD value of the control is 0.899, and the calculation result is:

$$\textbf{ATP (mmol/kg wet weight)} = \frac{(0.931-0.899)}{0.526-0.104} \times 1 \div 0.1 \times 0.9 \times 3$$

$$= 2.05 \text{ mmol/kg wet weight}$$

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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