Technical Manual

ATP Fluorimetric Assay Kit

- **Catalogue Code: MAES0002**
- **Size: 96T**
- **Research Use Only**

1. Key features and Sample Types

Detection method:
Chemiluminescence

Specification:
96T

Range:
0.003-5 µmol/L

Sensitivity:
0.003 µmol/L

Storage:
-20°C for 3 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 3 months.

Do not use components from different batches of kit.

2. Background

Adenosine 5' -triphosphate (ATP), an organic compound, is a natural nucleotide present in every cell consisting of purine base (adenine), ribose, and three phosphate groups. The content in tissue cells is generally in dynamic equilibrium, which is of great significance to constitute a stable energy supply environment inside the organism. ATP released 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 and cell samples.

4. Detection Principle

Under the catalyzation of luciferase, ATP react with luciferin and emits fluorescence, and the fluorescence intensity is proportional to the concentration of ATP within a certain range.

5. Kit components & storage

Item	Specification	Storage
Extracting Solution	50 mL × 2 vials	-20°C, 3 months
Standard Solution (100 μmol/L)	1 mL × 1 vial	-20°C, 3 months
Enzyme Reagent	2 vials Lyophilised	-20°C, 3 months, away from direct sunlight
Enzyme Diluent	14 mL × 1 vial	-20°C, 3 months
Black Microplate	96 wells	No requirement
Plate Sealer	2 pieces	

Materials required but not supplied

- Micropipettor
- Vortex mixer
- Water bath
- Centrifuge
- Chemiluminescence immunoassay analyzer or multifunctional microplate reader (with the function of detecting chemiluminescence)
- Tips (10 μL, 200 μL, 1000 μL)
- EP tubes (1.5 mL, 2 mL)
- Double distilled water

6. Assay Notes:

1. Dilute the samples to the optimal concentration for detection if the ATP content of samples exceed the detection range.

2. The sample size of each batch should be less than 30 (including standard wells).

3. Avoid the formation of bubbles when transferring the supernatant into the microplate.

4. It is recommended to aliquot the enzyme working solution into smaller quantities and store at -20°C. Avoid repeated freeze-thaw cycles.

7. Reagent Preparation

1. Bring extracting solution, 100μmol/L standard and enzyme diluent to room temperature, and place enzyme reagent on ice before detection.

2. Preparation of **enzyme working solution**: Dissolve 1 vial of enzyme reagent with 1 mL of enzyme diluent and mix fully. The prepared solution can be stored at -20˚C for a month when stored away from direct sunlight.

3. Preparation of **working solution:** Mix the enzyme working solution and enzyme diluent at a ratio of 1:5. Prepare fresh solution before use.

8. Sample Preparation

1. **Cell Sample:** Collect the cells and add extracting solution at a ratio of cells number (2×10^6): volume (mL) = 1:0.3. Then incubate in boiling water bath for 10 min, cool the tubes to room temperature with running water. Centrifuge at 10000 g for 10 min at 4°C, then take the supernatant for detection.

2. **Tissue sample:** Weigh the tissue accurately, cut into pieces, add 9 times the volume of extracting solution according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Then incubate in boiling water bath for 2 min, cool with the running water and centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice 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 (0.003-5 μmol/L).

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

Sample Type:	Dilution Factor:
10% Mouse heart tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse muscle tissue homogenate	1
10% Mouse liver tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse lung tissue homogenate	1

Note: The diluent of is extracting solution.

9. Assay Protocol

Ambient Temperature: 25-30°C

Plate Set Up:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	S1	S9	S17	S25	S33	S41	S49	S57	S65	S73
B	B	B	S2	S10	S18	S26	S34	S42	S50	S58	S66	S74
C	C	C	S3	S11	S19	S27	S35	S43	S51	S59	S67	S75
D	D	D	S4	S12	S20	S28	S36	S44	S52	S60	S68	S76
E	E	E	S5	S13	S21	S29	S37	S45	S53	S61	S69	S77
F	F	F	S6	S14	S22	S30	S38	S46	S54	S62	S70	S78
G	G	G	S7	S15	S23	S31	S39	S47	S55	S63	S71	S79
H	H	H	S8	S16	S24	S32	S40	S48	S56	S64	S72	S80

Note: A-H, standard wells; S1-S80, sample wells.

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10. Operation Steps

The preparation of standard curve

Dilute 100μmol/L standard solution with extracting solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.5, 1, 2, 2.5, 3, 4, 5 μmol/L.

The measurement of samples

1. **Standard well:** Add 100 μL of enzyme working solution into the corresponding well and stand for 5 min.
   Sample well: Add 100 μL of enzyme working solution into the corresponding well and stand for 5 min.

2. **Standard well:** Add 100 μL of standard with different concentrations into standard well, and mix fully immediately.
   Sample well: Add 100 μL of sample supernatant into sample well, and mix fully immediately.

3. Measure the fluorescence values of each well by the chemiluminescence immunoassay analyzer or multifunctional microplate reader.

Operation Table

	Standard well	Sample well
Working solution (μL)	100	100
Stand for 5 min.		
Standard with different concentrations (μL)	100	
Supernatant of sample (μL)		100
Mix fully immediately. Measure the fluorescence values of each well by the chemiluminescence immunoassay analyzer or multifunctional microplate reader.		

10. Calculations

Plot the standard curve by using fluorescence value (F) of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve with graph software (or EXCEL). The concentration of the sample can be calculated according to the formula based on the F value of sample. The standard curve is: y= ax + b.

1. Tissue sample:

$$\text{ATP content (μmol/kg wet tissue)} = (\Delta F - b) \div a \times f \div m \times V$$

2. Cell sample:

$$\text{ATP content (μmol/1} \times 10^9) = (\Delta F - b) \div a \times f \div n \times V$$

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y: $F_{Standard} - F_{Blank}$ (F_{Blank} is the fluorescence value when the standard concentration is 0
x: The concentration of Standard
a: The slope of standard curve
b: The intercept of standard curve
ΔF: The absolute fluorescence value of sample, $F_{Sample} - F_{Blank}$
f: Dilution factor of sample before tested
m: wet weight of sample, 0.05 g is recommended
V:The volume of homogenate medium during the preparation of tissue or cell sample, mL
N: The number of cells. For example, the number of cells is $5*10^6$, N is 5

11. Performance Characteristics

Detection Range	0.003-5 μmol/L
Sensitivity	0.003 μmol/L
Average recovery rate (%)	102
Average inter-assay CV (%)	6.5
Average intra-assay CV (%)	2.2

Analysis

For mouse lung tissue, take 0.05 g of fresh mouse lung sample, carry the assay according to the operation table.

The results are as follows:

Standard Curve: y = 26257 x + 1070, the average F value of the sample is 19512, the average F value of the blank is 92, and the calculation result is:

$$\begin{aligned}&\textbf{ATP content } (\boldsymbol{\mu mol/kg\ wet\ weight})\\&= \ (19512 - 92 - 1070) \div 26257 \div 0.05 \times 0.45\\&= 6.29\ \boldsymbol{\mu mol/kg\ wet\ weight}\end{aligned}$$

Safety Notes

Some of the reagents in the kit contain dangerous substances. Avoid 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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