PRODUCT INFORMATION & MANUAL

ATP Assay Kit (Colorimetric)

NBP3-24474

For research use only.
Not for diagnostic or therapeutic procedures.

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ATP Assay Kit (Colorimetric)

Catalog No: NBP3-24474

Method: Colorimetric method

Specification: 100Assays (Can detect 48 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.01 mmol/L

Detection range: 0.01-1.5 mmol/L

Average intra-assay CV (%): 3.6

Average inter-assay CV (%): 9.3

Average recovery rate (%): 103

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

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

▲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.

▲Detection principle

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

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Substrate	Powder × 2 vials	2-8℃ , 12 months
Reagent 3	Buffer Solution	24 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Enzyme Reagent	Powder × 2 vials	-20℃ , 12 months
Reagent 5	Protein Precipitator	6 mL × 1 vial	2-8℃ , 12 months
Reagent 6	Chromogenic Agent A	48 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 7	Chromogenic Agent B	16 mL × 1 vial	2-8℃ , 12 months
Reagent 8	Stop Solution	60 mL× 1 vial	2-8℃ , 12 months
Reagent 9	Standard	Powder × 4 vials	2-8℃ , 12 months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲Materials prepared by users

Instruments

Spectrophotometer (636 nm), Micropipettor, Incubator, Water bath, Vortex mixer, Centrifuge

Reagents

Double distilled water, Normal saline (0.9% NaCl)

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the 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.

▲Precautions

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

▲The key points of the assay

1. The fresh samples should be used.
2. Avoid phosphorus pollution is the key for assay, it is recommended to use disposable test tubes.
3. It is recommended to prepare 2%~5% tissue homogenate for assay.

Pre-assay preparation

▲Reagent preparation

1. Preparation of reagent 2 application solution:

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

2. Preparation of reagent 4 application solution:

Dissolve a vial of reagent 4 fully with 1.8 mL of double distilled water fully. The prepared solution can be stored at -20℃ for 7 days.

3. Preparation of control working solution:

Mix the reagent 2 application solution, reagent 3, double distilled water at the ratio of 100:200:30 fully. Prepare the needed amount fresh solution before use.

4. Preparation of detection working solution:

Mix the reagent 2 application solution, reagent 3, reagent 4 application solution at the ratio of 100:200:30 fully. Prepare the needed amount fresh solution before use.

5. Preparation of chromogenic agent:

Mix the reagent 6 and reagent 7 at the ratio of 3:1 fully. Place it at 37℃ for 1 hour. Prepare the needed solution before use.

6. Preparation of 10 mmol/L ATP standard stock solution:

Dissolve a vial of reagent 9 with 1 mL of double distilled water fully. The prepared solution can be stored at -20℃ for 7 days.

7. Preparation of 1 mmol/L ATP standard application solution:

Dilute 10 mmol/L ATP standard stock solution with double distilled water for 10 times. The prepared solution can be stored at -20℃ for 7 days.

▲Sample preparation

Sample requirements

The fresh samples should be used.

Tissue sample:

Weigh the tissue accurately, cut into pieces, then adding 9 times of the volume of reagent 1 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.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and 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	2-3
10% Rat liver tissue homogenate	2-3
10% Rat brain tissue homogenate	2-3
10% Rat kidney tissue homogenate	2-3
10% Rat lung tissue homogenate	2-3

Note: The diluent is double distilled water.

Assay protocol

▲Detailed operating steps

1. Blank tube: Take 30 μL of 1 mmol/L ATP standard application solution to the 1.5 mL EP tube, then add 330 μL of control working solution.
   Standard tube: Take 30 μL of 1 mmol/L ATP standard application solution to the 1.5 mL EP tube, then add 330 μL of detection working solution.
   Control tube: Take 30 μL of sample to the 1.5 mL EP tube, then add 330 μL of control working solution.
   Sample tube: Take 30 μL of sample to the 1.5 mL EP tube, then add 330 μL of detection working solution.
2. Mix fully and incubate at 37℃ for 30 min.
3. Add 50 μL of reagent 5 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 reagent 8 to each tube.
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.
   Note: cuvettes should be washed with tap water for 10 times, then washed with double distilled water for 4~5 times, avoid contaminated with phosphorus.

▲Summary operation table

	Blank tube	Standard tube	Control tube	Sample tube
1 mmol/L ATP standard application solution (μL)	30	30		
Sample(μL)			30	30
Control working solution (μL)	330		330	
Detection working solution (μL)		330		330
Mix fully and incubate at 37℃ for 30 min.				
Reagent 5	50	50	50	50
Mix fully and centrifuge 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				
Reagent 8 (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 at 636 nm.				

▲Calculation

Tissue sample

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

Note:

c: Concentration of standard (1 mmol/L)

m: The fresh weight of tissue sample (g).

V_1: The volume of reagent 1 in the sample preparation step of tissue sample.

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

Take rat muscle tissue, treat the sample according to the manual, then dilute the sample with double distilled water for 3 times and carry the assay according to the operation table. 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:

$$\text{ATP (mmol/kg fresh weight)} = \frac{(0.761 - 0.758)}{(0.622 - 0.048)} \times 1 \div 0.1 \times 0.9 \times 3 = 0.14 \text{ mmol/kg fresh weight}$$

Appendix II References

1. Agteresch H J, Dagnelie P C, Berg J W v d, et al. Adenosine Triphosphate established and potential clinical applications[J]. Drugs, 1999, 58(2): 211-232.
2. Burnstock G. Adenosine Triphosphate [J]. Encyclopedia of Neuroscience, 2009, 23(52): 105-113.