PRODUCT INFORMATION & MANUAL

ATP Assay Kit (Colorimetric)

NBP3-24473

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

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ATP Assay Kit (Colorimetric)

Catalog No: NBP3-24473

Method: Colorimetric method

Specification: 96T (Can detect 46 samples without duplication)

Measuring instrument: Microplate reader

Sensitivity: 0.01 mmol/L

Detection range: 0.03-1.5 mmol/L

Average intra-assay CV (%): 5.1

Average inter-assay CV (%): 5.4

Average recovery rate (%): 94

- ▲ 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 animal 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. The content of phosphocreatine was determined by colorimetric method to reflect the content of ATP.

▲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	12 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 7	Chromogenic Agent B	4 mL × 1 vial	2-8℃ , 12 months
Reagent 8	Stop Solution	12 mL× 1 vial	2-8℃ , 12 months
Reagent 9	Standard	Powder × 4 vials	2-8℃ , 12 months
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
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

Microplate reader (630-640 nm), Micropipettor, Tubes, Vortex mixer, Incubator, 100℃ Water bath, Centrifuge

Reagents

Double distilled water

⚠ 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. When the OD value is more than 1, it is necessary to increase the dilution ratio and detect again
4. Bring all other reagents to room temperature before use, except for reagent 3, which should be placed on ice for detectiom.

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. 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

1. 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 (0.03-1.5 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.

Assay protocol

▲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.

▲Detailed operating steps

1. Enzymatic reaction

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 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℃ 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 supernatant of each tube for detection.

2. 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 5 s with microplate reader and stand for 2 min at room temperature.

4) Add 100 μL of reagent 8 to each well.

5) Mix fully for 5 s with microplate reader, stand at room temperature for 5 min, and measure the OD value of each well at 636 nm.

▲Summary operation table

Enzymatic reaction

	Blank tube	Standard tube	Control tube	Sample tube
1 mmol/L ATP standard solution (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℃ for 30 min.				
Reagent 5	50	50	50	50
Mix fully and centrifuge 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				
Reagent 8 (μL)	100	100	100	100
Mix fully, stand at room temperature for 5 min, and measure the OD value at 636 nm.				

▲Calculation

Tissue sample:

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

Note:

c: Concentration of standard (1 mmol/L)

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

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

f: Dilution factor of sample before test.

Appendix I Data

▲Example analysis

For crucian muscle tissue, dilute 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 0.104, 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:

ATP content (mmol/kg wet weight)

= (0.931-0.899) ÷ (0.526-0.104) ×1 ÷0.1×0.9×3

=2.05 mmol/kg wet 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.