PRODUCT INFORMATION & MANUAL

ATPase Activity Assay Kit (Colorimetric)

NBP3-24475

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

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ATPase Activity Assay Kit (Colorimetric)

Catalog No: NBP3-24475

Method: Colorimetric method

Specification: 96T (Can detect 40 samples without duplication)

Instrument: Microplate reader

Sensitivity: 0.06 U/L

Detection range: 0.06 - 2.75 U/L

Average intra-assay CV (%): 4

Average inter-assay CV (%): 5

Average recovery rate (%): 95

▲ 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 measure ATPase activity in animal tissue and cell samples.

▲Detection principle

ATPase, also known as adenosine triphosphate, is an important high-energy compound in organisms, which can maintain the balance of cell membrane potential and ions.

ATPase catalyzes the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and inorganic phosphorus. The activity of ATPase was determined by inorganic phosphorus production per unit time.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Buffer Solution	30 mL × 1 vial	2-8℃ , 12 months
Reagent 2	Substrate	Powder × 1 vial	2-8℃ , 12 months, shading light
Reagent 3	Acid Reagent	25 mL × 1 vial	2-8℃ , 12 months
Reagent 4	Chromogenic Agent A	12 mL × 1 vial	2-8℃ , 12 months, shading light
Reagent 5	Chromogenic Agent B	4 mL × 1 vial	2-8℃ , 12 months
Reagent 6	10 mmol/L Standard	0.5 mL × 1 vial	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

Vortex mixer, Incubator, Centrifuge, Microplate reader (630-640 nm, optimum wavelength: 640 nm)

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. To avoid external phosphorus contamination, it is recommended to wash the experimental apparatus for 10 times.
2. When the OD value is more than 0.5, it is necessary to increase the dilution ratio and detect again.
3. The fresh samples should be used.

Pre-assay preparation

▲ Reagent preparation

1. Bring all reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dissolve a vial of reagent 2 with 10 mL double distilled water and mix fully. The prepared solution can be stored at 2-8℃ for 7 days with shading light.
3. Preparation of chromogenic working solution:
   Mix the reagent 4 and reagent 5 at the ratio of 3:1 fully, stand at 37℃ for 1 h. Prepare the fresh reagent before use. The prepared solution should be stored with shading light and should be used within 10 h.
4. Preparation of 0.1 mmol/L standard:
   Dilute reagent 6 with double distilled water at a ratio of 1:99. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C.

▲ Sample preparation

1. Tissue sample:

Accurately weigh the tissue, add normal saline (0.9% NaCl) at a ratio of Weight (g): Volume (mL) =1:9 and homogenize the sample in ice water bath. Then centrifuge at 10000 g for 15 min, then take the supernatant for detection. If the supernatant is turbidity after centrifugation, repeated centrifuge until clear before use. Meanwhile, determine the protein concentration of supernatant.

2. Cell sample:

Collect the cells and wash the cells with PBS (0.01 M, pH 7.4) for 1~2 times. Centrifuge at 1000 g for 10 min and then discard the supernatant and keep the cell sediment. Add homogenization medium at a ratio of cell number (10^6): normal saline (0.9% NaCl) (mL) =5: 1, Sonicate or mechanical homogenate in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant for detection. Meanwhile, determine the protein concentration of supernatant.

▲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 (0.06 - 2.75 U/L).

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

Sample type	Dilution factor
10% Rat liver tissue homogenate	5-10
10% Rat kidney tissue homogenate	5-10
10% Rat heart tissue homogenate	5-10
10% Rat spleen tissue homogenate	5-10
10% Rat lung tissue homogenate	5-10
10% Mouse liver tissue homogenate	5-10
10% Mouse spleen tissue homogenate	5-10
10% Mouse lung tissue homogenate	5-10
10% Mouse kidney tissue homogenate	5-10
293T cell	1

Note: The diluent is normal saline (0.9%NaCl).

Assay protocol

▲Plate set up

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

Note: A-H, standard wells; S1-S40, control wells; S1'-S40', sample wells.

▲Detailed operation steps

1. The preparation of standard curve

Dilute 0.1 mmol/L standard solution with double distilled water to a serial concentration. The recommended dilution gradient is as follows: 0.00, 0.01, 0.02, 0.03, 0.05, 0.06, 0.08, 0.10 mmol/L. Reference is as follows:

Number	Standard concentrations (mmol/L)	0.1 mmol/L standard solution (μL)	Double distilled water (μL)
A	0.00	0	200
B	0.01	20	180
C	0.02	40	160
D	0.03	60	140
E	0.05	100	100
F	0.06	120	80
G	0.08	160	40
H	0.10	200	0

2. The measurement of samples

Enzymatic reaction

(1) Control tube: Take 160 μL of double distilled water to the 1.5 mL EP tube.

Sample tube: Take 160 μL of double distilled water to the 1.5 mL EP tube.

(2) Add 100 μL of sample to the sample tube.

(3) Add 260 μL of regent 1 to each tube.

(4) Add 80 μL of regent 2 working solution to each tube.

(5) Mix fully and incubate at 37℃ for 30 min.

(6) Add 100 μL of reagent 3 to each tube and mix fully.

(7) Add 100 μL of sample to the control tube. Mix fully and centrifuge at 3500 g for 10 min, then take the supernatant for detection.

Chromogenic reaction

(1) Standard well: Add 30 μL of standard solution with different concentrations to the corresponding wells.

Control well: Add 30 μL of supernatant from control tube to the corresponding wells.

Sample well: Add 30 μL of the supernatant from sample tube to the corresponding wells.

(2) Add 100 μL of chromogenic working solution to each well.

(3) Mix fully with microplate reader for 5 s and incubate at room temperature for 2 min.

(4) Add 100 μL of reagent 3 to each well.

(5) Mix fully with microplate reader for 5 s and incubate at room temperature for 10 min with shading light. Measure the OD value of each well at 640 nm with microplate reader.

▲Summary operation table

Incubation reaction

	Control tube	Sample tube
Double distilled water (μL)	160	160
Sample (μL)		100
Regent 1 (μL)	260	260
Regent 2 working solution (μL)	80	80
Mix fully and incubate at 37℃ for 30 min.		
Regent 3 (μL)	100	100
Sample (μL)	100	
Mix fully and centrifuge at 3500 g for 10 min, then take the supernatant for detection.		

Chromogenic reaction

	Standard well	Control well	Sample well
Standard solution with different concentrations (μL)	30		
Supernatant of sample (μL)		30	30
Chromogenic working solution (μL)	100	100	100
Mix fully and incubate at room temperature for 2 min			
Reagent 3 (μL)	100	100	100
Mix fully and incubate at room temperature for 10 min with shading light. Measure the OD value of each well.			

▲Calculation

Plot the standard curve by using OD value 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 OD value of sample. The standard curve is: y= ax + b.

tissue and cell sample:

Definition: The amount of ATPase in 1 g tissue or cell protein per 1 minute that hydrolyze the substrate to produce 1 μmol of product at 37°C is defined as 1 unit.

$$\text{ATPase activity (U/gprot)} = (\Delta A - b) \div a \div C_{pr} \div T \times f \times 1000^*$$

Note:

y: $OD_{Standard} - OD_{Blank}$ (OD_{Blank} is the change of OD value when the standard concentration is 0).

x: The concentration of standard.

a: The slope of standard curve.

b: The intercept of standard curve.

ΔA: $OD_{Sample} - OD_{Control.}$

T: The time of incubation reaction, 30 min.

C_{pr}: The concentration of protein in sample, gprot/L.

f: Dilution factor of sample before test.

1000*:1 mmol/L = 1000 μmol/L

Appendix I Data

▲Example analysis

For rat liver tissue, take 10% rat liver tissue homogenate, dilute for 5 times, and carry the assay according to the operation table.

The results are as follows:

standard curve: y = 5.5215 x – 0.0044, the OD value of the control is 0.639, the OD value of the sample is 0.784, the concentration of protein in sample is 7.75 gprot/L, and the calculation result is:

ATPase activity (U/gprot) = (0.784 - 0.639 + 0.0044) ÷ 5.5215 ÷ 7.75 ÷ 30 × 1000 × 5 = 0.62 U/gprot

Detect 10% rat liver tissue homogenate (the concentration of protein is 7.75 gprot/L, dilute for 5 times), 10% rat kidney tissue homogenate (the concentration of protein is 6.85 gprot/L, dilute for 5 times), 10% rat heart tissue homogenate (the concentration of protein is 4.80 gprot/L, dilute for 5 times) and 10% mouse liver tissue homogenate (the concentration of protein is 9.05 gprot/L, dilute for 5 times), according to the protocol, the result is as follows: