PRODUCT INFORMATION & MANUAL

ATP Assay Kit (Fluorometric)

NBP3-24472

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

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ATP Assay Kit (Fluorometric)

Catalog No: NBP3-24472

Method: Chemiluminescence immunoassay analyzer, Multifunctional microplate reader

Specification: 96T (Can detect 80 samples without duplication)

Measuring instrument: Chemiluminescence immunoassay analyzer, Multifunctional microplate reader

Sensitivity: 0.003 μmol/L

Detection range: 0.003-5 μmol/L

Detection range: 0.01-0.83 U/L

Average intra-assay CV (%): 2.2

Average inter-assay CV (%): 6.5

Average recovery rate(%): 102

▲ 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 and cell samples.

▲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.

▲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.

▲Kit components & Storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	50 mL × 2 vials	-20℃ , 12 months
Reagent 2	100 μmol/L Standard Solution	1 mL × 1 vial	-20℃ , 12 months
Reagent 3	Enzyme Reagent	Power × 2 vials	-20℃ , 12 months, shading light
Reagent 4	Enzyme Diluent	14 mL × 1 vial	-20℃ , 12 months
	Black 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, Centrifuge, Water bath , Chemiluminescence immunoassay analyzer or multifunctional microplate reader (with the function of detecting chemiluminescence).

▲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. 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. Prevent the formulation of bubbles when the supernatant is transferred into the microplate.
4. It is recommended to aliquot the reagent 3 working solution into smaller quantities and store at -20℃ . Avoid repeated freeze-thaw cycles.

Pre-assay preparation

▲Reagent preparation

1. Bring reagent 1, reagent 2, reagent 4 to room temperature, and place reagent 3 on ice before detection.
2. Preparation of reagent 3 working solution:
   Dissolve 1 vial of reagent 3 powder with 1 mL of reagent 4 and mix fully. The prepared solution can be stored at -20°C for a month with shading light.
3. Preparation of enzyme working solution:
   Mix the reagent 3 working solution and reagent 4 at a ratio of 1:5. Prepare the needed fresh solution before use.

▲Sample preparation

Tissue sample

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

Cells sample

Collect the cells and add reagent 1 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.

▲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 (0.003-5 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 is reagent 1.

Assay protocol

▲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.

▲Detailed operation steps

The preparation of standard curve

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

Number	Standard concentrations (μmol/L)	100 μmol/L standard solution (μL)	Reagent 1 (μL)
A	0	0	1000
B	0.5	5	995
C	1	10	990
D	2	20	980
E	2.5	25	975
F	3	30	970
G	4	40	960
H	5	50	950

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.

▲Summary operation table

	Standard well	Sample well
Enzyme 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.		

▲Calculation

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.

For tissue sample:

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

For cells sample:

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

Note:

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.

Appendix I Data

▲Example analysis

For mouse lung tissue, take 0.05 g of fresh mouse lung sample and 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:

$$\text{ATP content (μmol/kg wet weight)} = \frac{(19512 - 92 - 1070)}{26257} \div 0.05 \times 0.45$$

$$= 6.29\ \text{μmol/kg wet weight}$$

Appendix II References

1. Agteresch H J, Dagnelie P C, Berg J W, 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.