ATP Assay Kit (Enzymatic Method)

A1508987

Storage -20℃. Store in the dark.

Shipping Shipped with ice packs. Please store under the specified storage conditions immediately upon receipt.

Introduction

ATP is a core molecule in cellular energy metabolism, and its content directly reflects the energy status of organisms and their organs. As a key energy substance, ATP participates in and regulates various physiological and pathological activities of cells, and fluctuations in its levels significantly affect cellular functions. Under normal conditions, ATP levels decrease during apoptosis, necrosis, or toxic states; conversely, under specific stimuli such as high glucose, ATP levels may increase in certain cells. A decline in ATP often indicates impaired mitochondrial function, and during apoptosis, it is frequently accompanied by a reduction in mitochondrial membrane potential.

This kit can be used to measure ATP content in animal tissue or cell samples. Hexokinase (HK) catalyzes the reaction between glucose and ATP to produce glucose-6-phosphate (G6P). Glucose-6-phosphate dehydrogenase (G6PD) further catalyzes the dehydrogenation of G6P to generate NADPH. NADPH then reacts with a chromogenic agent, and the resulting product exhibits a characteristic absorption peak at 460 nm, allowing the determination of ATP content.

Kit Contents

A1508987	Component	96 T	Storage conditions
A1508987A	Lysis Buffer	100 mL	-20°C
A1508987B	Enzyme Reagent A	1 mL	-20°C. Store in the dark.
A1508987C	Enzyme Reagent B	5 mL	-20°C. Store in the dark.
A1508987D	Enzyme Reagent C	5 mL	-20°C. Store in the dark.
A1508987E	WST-8	250 μL	-20°C. Store in the dark.
A1508987F	1-mPMS	250 μL	-20°C. Store in the dark.
A1508987G	Reaction Buffer	10 mL	-20°C
A1508987H	0.5mM Standard	0.6 mL	-20°C. Store in the dark.

Instruction for use

1. Reagent Preparation

(1) Before the assay, thaw all reagents completely. After thawing, keep 1-mPMS at room temperature until use, and place the remaining reagents on ice until use.

(2) Preparation of Enzyme Working Solution A:

Mix Enzyme Reagent A and Reaction Buffer at a volume ratio of 1:4 to prepare Enzyme Working Solution A. It is recommended to prepare fresh before use. Any unused portion can be temporarily stored at -20°C.

(3) Preparation of Chromogenic Working Solution (example for 1 mL):

After fully thawing WST-8 and 1-mPMS, transfer 50 μL of each into an EP tube, add 900 μL of Reaction Buffer, and mix thoroughly. Each reaction well requires 50 μL of Chromogenic Working Solution. Prepare the required volume accordingly and use immediately.

(4) Preparation of 40 μmol/L Standard Solution:

Mix 0.5 mM ATP Standard and Lysis Buffer at a volume ratio of 1:11.5. Prepare the required volume as needed. The unused 40 μmol/L Standard Solution can be stored at -20°C for one month.

(5) Dilution of Standard Solutions to Different Concentrations:

No.	①	②	③	④	⑤	⑥	⑦	⑧
Standard concentration (μmol/L)	0	5	10	15	20	25	30	40
40 μmol/L Standard (μL)	0	100	200	300	400	500	600	800
Lysis Buffer	800	700	600	500	400	300	200	0

2. Sample Preparation

(1) For adherent cells:

Remove the culture medium. Add Lysis Buffer to the cells at a volume of 200 μL per well of a 6-well plate (i.e., 1/10 of the culture medium volume of 2 mL). To ensure complete lysis, pipette repeatedly or shake the plate so that the Lysis Buffer fully contacts and lyses the cells. Cells typically lyse immediately upon contact with the Lysis Buffer. After lysis, centrifuge at 12,000× g for 5 minutes at 4°C. Transfer the supernatant to ice (or an ice box) for subsequent measurement.

(2) For suspension cells:

Pellet the cells by centrifugation in a tube, discard the supernatant, and gently tap the tube to disperse the cell pellet. Add Lysis Buffer at a volume of 200 μL per well-equivalent of a 6-well plate. To ensure complete lysis, tap the bottom of the tube or vortex to allow the Lysis Buffer to fully contact and lyse the cells. Cells typically lyse immediately upon contact with the Lysis Buffer. After lysis, centrifuge at 12,000×g for 5 minutes at 4°C. Transfer the supernatant to ice (or an ice box) for subsequent measurement.

(3) For tissue samples:

Take fresh tissue samples and rinse with cold PBS to remove impurities. Add Lysis Buffer at a

ratio of tissue mass (g) to Lysis Buffer volume (mL) of 1:9 (e.g., for 0.1 g of tissue sample, add 0.9 mL of Lysis Buffer), and perform mechanical homogenization at 4°C. Place the resulting tissue homogenate in a boiling water bath for 3 minutes, then cool under running water to room temperature (25°C). Centrifuge at 10,000×g for 10 minutes at 4°C, and transfer the supernatant to ice for subsequent determination.

(4) Preliminary sample test:

Before the formal assay, it is recommended to select 2-3 samples with expected large differences, dilute them to different concentrations, and perform a preliminary test.

3. ATP Concentration Determination

(1) Standard wells: Add 100 μL of each standard solution at different concentrations into the corresponding wells of the plate. Then sequentially add 50 μL of Enzyme Working Solution A, 50 μL of Enzyme Reagent B, and 50 μL of Chromogenic Working Solution to each well.

(2) Sample wells: Add 100 μL of sample into the corresponding wells. Then sequentially add 50 μL of Enzyme Working Solution A, 50 μL of Enzyme Reagent B, and 50 μL of Chromogenic Working Solution to each well.

(3) Control wells: Add 100 μL of sample into the corresponding wells. Then sequentially add 50 μL of Enzyme Working Solution A, 50 μL of Enzyme Reagent C, and 50 μL of Chromogenic Working Solution to each well.

(4) Shake the plate for 1-2 minutes to mix, then incubate at 37°C for 15 minutes. Measure the OD of each well at 460 nm using a microplate reader.

4. Result Calculation

(1) Standard curve fitting: $Y = aX + b$

Formula for ATP content in cell samples:

ATP content (μmol/L) = $(\Delta A - b) \div a \times f$

Formula for ATP content in tissue samples:

ATP content (μmol/kg wet weight) = $(\Delta A - b) \div a \times f \div m \times V$

Explanation:

Y = OD value of standard well - OD value of blank well (OD value when standard concentration is 0);

X = Standard concentration;

a = Slope of the standard curve;

b = Intercept of the standard curve;

ΔA = OD value of sample well - OD value of control well;

f = Dilution factor of the sample before addition to the detection system;

m = Sample mass (e.g., 0.1 g);

V = Volume of Reagent 1 added during tissue homogenization (e.g., 0.9 mL).

(2) Performance characteristics:

This kit exhibits good linearity in the range of 0-40 μmol/L (example shown). The sensitivity is 0.5 μmol/L.

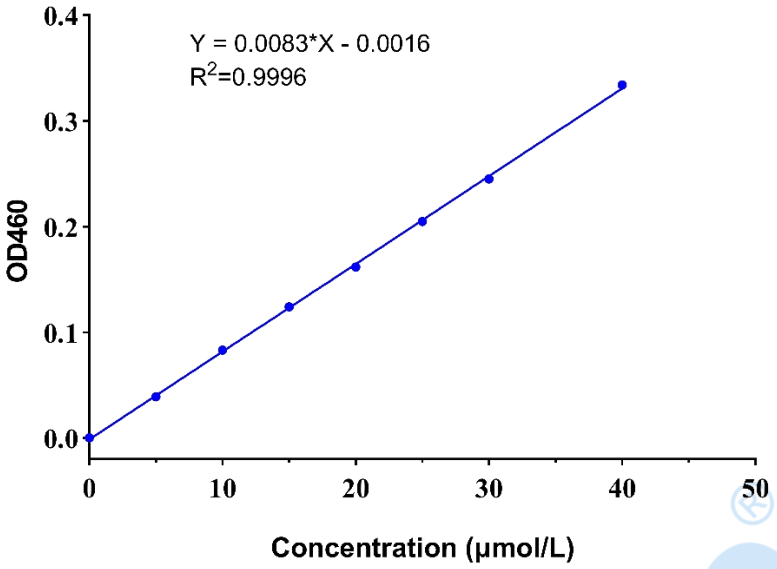

Fig. 1. Example Standard Curve

Precautions

1. This kit is for research use only. If it is used for clinical diagnosis or any other purpose, our company shall not be held responsible for any resulting issues, nor assume any legal liability.
2. Please read the instructions carefully before the experiment and calibrate the instruments accordingly. Strictly follow the instructions during the experiment.
3. Wear a lab coat and latex gloves during the experiment for proper protection.
4. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the analyte concentration in the sample is too high or too low, dilute or concentrate the sample appropriately.
5. If the sample to be tested is not among the sample types listed in the instructions, it is recommended to perform a preliminary experiment to verify the validity of the detection.
6. The final experimental results are closely related to factors such as the validity of the reagents, the operator's technique, and the experimental environment. Our company is only responsible for the kit itself and shall not be liable for sample consumption caused by the use of the kit. Before use, please fully consider the possible amount of sample to be used and reserve sufficient sample.