ATP Content Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: High performance liquid chromatography

Catalog Number: BC0304

Sizes： 50T/48S

Components:

Extract solution I: 80 mL×1. Storage at 4°C.

Extract solution II： 40 mL×1. Storage at 4°C.

Reagent I: 15 mL×1. Storage at 4°C. Before use, take 3.5 mL of reagent I and add it to 1000 mL of ultrapure water, adjust its pH to 6.15 with reagent II to form mobile phase B, and seal it.

Reagent II:10 mL ×1. Storage at 4°C.

Standard： Powder×1. Storage at 4°C.

Product Description：

Adenosine triphosphate (ATP) is considered to be a universal energy source that is essential for cell synthesis in the survival and reproduction of all organisms. ATP can be produced through a variety of cellular pathways. The most typical example is synthesis by adenosine triphosphate synthase through oxidative phosphorylation in mitochondria, or synthesis by photosynthesis in plant chloroplasts. The main energy sources for ATP synthesis are glucose and fatty acids.

ATP has an absorption peak at 254 nm, and its content can be determined by high performance liquid chromatography.

Reagents and Equipment Required but Not Provided：

High-efficiency liquid chromatograph (C18 column (4.6×250 mm), ultraviolet detector (VWD)), desktop centrifuge, adjustable pipette, mortar/ homogenizer, brown EP tube, 50 syringe filters (water, 0.45 μm), syringe, suction filter, filter membrane (organic, water), 50 brown injection bottle (2 mL), acetonitrile (chromatographically pure, 500 mL), ultrapure water.

Preparations before the experiment:

1. Use 500 mL of chromatographically pure acetonitrile (mobile phase A) and 1000 mL of configured mobile phase B to filter with a filter membrane to remove impurities in the solvent to prevent clogging the chromatographic column. (Acetonitrile uses 0.45 μm organic filter membrane for suction filtration, and the configured mobile phase B uses 0.22 μm aqueous filter membrane for suction filtration).

2. Ultrasound the prepared mobile phases A and B for 30 minutes to remove the gas in the solvent to prevent clogging the chromatographic column and affecting the experimental results.

3. Preparation of standard: 1 μmol/mL ATP-Na2 standard solution is diluted with distilled water to 0.5 μmol/mL, 0.1 μmol/mL, 0.05 μmol/mL, 0.01 μmol/mL, 0.005 μmol/mL ATP standard solution. (The prepared standard concentration is for reference only and can be adjusted according

to the actual sample concentration). The standard is filtered use an aqueous syringe filter into the brown injection bottle to be tested (please place it at room temperature before testing, so as not to affect the retention time).

Procedure

I. Sample preparation:

(1) Tissue sample: According to the ratio of tissue (g): extract solution I (mL) = 1:5~10 (it is recommended to weigh 0.3 g tissue sample and add 1.5 mL extract solution I) to add extract solution I, homogenate on ice, and extract in an ice bath for 40 min. Centrifuge at 10000 rpm for 10 min at 4°C, take 750μL of the supernatant, add 750μL of extract II, shake well (5 min) and mix well, centrifuge again at 10000 rpm at 4°C for 10 min, and take the supernatant to filter with an aqueous syringe filter into the brown injection bottle to be tested at room temperature (within 2 h).

(2) Cell sample: According to the ratio of 10 million cell (units): extract solution I (mL)= 1000~500:1 (it is recommended to take 10 million cell samples and add 1 mL extract solution I) add extraction solution I, ultrasonic breaking Cells on ice (power 300W, ultrasound for 3 seconds, interval of 7 seconds, total time of 3 minutes); centrifuge at 4℃, 10000 rpm for 10 minutes, take 750μL of the supernatant, add 750μL of extract II, shake well (5 min) and mix well, centrifuge again at 10000 rpm at 4°C for 10 min, and take the supernatant to filter with an aqueous syringe filter into the brown injection bottle to be tested at room temperature (within 2 h).

(3) Serum: It is recommended to take 0.4 mL serum sample, add 0.6 mL extraction solution 1, and extract for 40 min in ice bath. Centrifuge at 10000 rpm for 10 min at 4°C, take 750μL of the supernatant, add 750μL of extract II, shake well (5 min) and mix well, centrifuge again at 10000 rpm at 4°C for 10 min, and take the supernatant to filter with an aqueous syringe filter into the brown injection bottle to be tested at room temperature (within 2 h).

II. Determination procedure：

1. Turn on the computer, turn on the switch buttons of each module of the HPLC, install the chromatographic column, open the software, and set the injection volume in the method group to 10 μL, column temperature: 27°C, flow rate 0.8 mL/min, and wavelength 254 nm, the elution program is as shown in the table below, and the sampling time is 70 min. After setting, save the method group.
2. Clean the column with the mobile phase, equilibrate the column with a mobile phase ratio of acetonitrile: mobile phase B (pH = 6.15) = 2: 98, and start the injection after the baseline is stable.
3. Detect the prepared standard solution, the injection volume is 10 μL, the ATP can be separated within 10 minutes, and the retention time of ATP is about 7.8 min (the pH of the system, column, mobile phase, etc. are different, the retention time is different, only reference).
4. Detect the prepared sample solution, the injection volume is 10 μL, and detect the peak area of ATP at the corresponding retention time.

Time	Mobile Phase	
	A	B
0 min	2%	98%
10 min	2%	98%
15 min	70%	30%
50 min	70%	30%
55 min	2%	98%
70 min	2%	98%

III. Calculations：

I. Draw a standard curve of ATP with the standard concentration (μmol/mL) as x and the peak area as the y. Substitute the peak area of the sample into the standard curve to calculate the ATP concentration x (μmol/mL) in the sample.

II. ATP content caculation

A. Sample weight

ATP (μmol/g)＝$2x \times V_E \div W = 3x \div W$

ATP (μg/g）$= 2x \times V_E \times 507.18 \div W = 1521.54x \div W$

B. Cell amount

ATP (μmol/10^4 cell)＝$2x \times V_E \div$ number of cells

ATP (μg/g10^4 cell）$= 2x \times V_E \times 507.18 \div$ number of cells

C. Liquid volume:

ATP (μmol/mL)＝$2x \times V_E \div V_S = 5x$

ATP (μg/mL）$= 2x \times V_E \times 507.18 \div V_S = 2535.9x$

V_E: volume of extract solution I, 1.5 mL; W: Sample weight, g; M_{ATP}: 507.18; V_S： volume of sample； 2: Sample dilution factor.

Note:

Precautions:

1. After the detection, the chromatographic column needs to be flushed with high-concentration ultrapure water (about 20-30 column volumes) to prevent clogging the chromatographic column. Finally, flush the column according to the specifications of the column to prevent damage to the chromatographic column.

2. The dilution factor of the standard should be determined according to the concentration of ATP in

the sample. The peak area of ATP in the sample must be within the peak area of the standard solution of different concentrations. The dilution factor of the standard is only a reference. If the ATP concentration in the sample is too high, it is recommended to dilute it before testing.

3. ATP, especially the ATP in the sample after extraction is not stable at room temperature, so it needs to be operated as soon as possible.

4. If the sample number is too large, it is recommended to test the standard solution once a day (one standard solution is sufficient) to determine the corresponding retention time.