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Nucleotide（ATP、ADP、AMP） Content HPLC Assay Kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number: AK0658-50T-48S

Sizes： 50T/48S

Components:

Extract solution I: 80 mL×1. Storage at 2-8℃.

Extract solution II： 40 mL×1. Storage at 2-8℃.

Reagent I: 15 mL×1. Storage at 2-8℃. 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 2-8℃.

ATP Standard： Powder×1. Storage at -20℃. Before use, 1.8 mL distilled water is added to prepare 1 μmol / mL ATP standard solution, which was frozen at -20 °C. In order to ensure the integrity of ATP, avoid repeated freezing and thawing.

ADP Standard： Powder×1. Storage at -20℃. Before use, 2.34 mL distilled water is added to prepare 1 μmol / mL ATP standard solution, which was frozen at -20 °C. In order to ensure the integrity of ATP, avoid repeated freezing and thawing.

AMP Standard： Powder×1. Storage at -20℃. Before use, 2.0 mL distilled water is added to prepare 1 μmol / mL ATP standard solution, which was frozen at -20 °C. In order to ensure the integrity of ATP, avoid repeated freezing and thawing.

Product Description：

Nucleotides have important biological functions. They are a class of compounds composed of three substances: purine base or pyrimidine base, ribose or deoxyribose, and phosphoric acid. They are mainly involved in the formation of nucleosides.

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.

Adenosine diphosphate (ADP) is widely present in animals, plants, microorganisms and cultured cells. In organisms, ADP is a product of breaking a high-energy phosphate bond (ATP) ydrolyzed to lose a phosphate radical) and releasing energy.

Adenosine monophosphate (AMP) is widely found in animals, plants, microorganisms and cultured cells. It is formed after ATP and ADP release energy in the body. It can continue to bind phosphate groups to form adenosine diphosphate (ADP) and adenosine triphosphate (ATP). It is the product of incomplete

hydrolysis of ATP.

ATP、ADP、AMP have 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 **ATP standard:** 1 μmol/mL ATP 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).

3. Preparation of **ADP standard:** 1 μmol/mL ADP 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 ADP 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).

3. Preparation of **AMP standard:** 1 μmol/mL AMP 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 AMP 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, ADP , AMP can be separated within 10 minutes, and the retention time of ATP, ADP , AMP is about 7.8 min, 6.7min and 5.4min (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, ADP, AMP 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 standard curves of ATP, ADP, AMP 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, ADP, AMP concentration x_1, x_2, x_3 (μmol/mL) in the sample.

II. ATP content calculation

A. Sample weight

ATP (μmol/g)＝$2\ x_1 \times V_E \div W = 3\ x_1 \div W$

ATP (μg/g）=$2\ x_1 \times V_E \times 551.14 \div W = 1653.42\ x_1 \div W$

V_E: volume of extract solution I, 1.5mL; W: Sample weight, g; M_{ATP}: 551.14; 2: Sample dilution factor.

B. Liquid volume:

ATP (μmol/mL)＝$2\ x_1 \times V_E \div V_S = 5 \times x_1$

ATP (μg/mL）=$2\ x_1 \times V_E \times 551.14 \div V_S = 2755.7 \times x_1$

V_E: volume of extract solution I, 1mL; M_{ATP}: 551.14; V_S： volume of sample, 0.4mL； 2: Sample dilution factor.

C. Cell amount

ATP (μmol/10^4 cell)＝$2\ x_1 \times V_E \div N = 2 \times x_1 \div N$

ATP (μg/g10^4 cell）=$2\ x_1 \times V_E \times 551.14 \div N = 1102.28 \times x_1 \div N$

V_E: volume of extract solution I, 1mL; M_{ATP}: 551.14; N: number of cells, 10^4 as a unit; 2: Sample dilution factor.

III. ADP content calculation

A. Sample weight

ADP (μmol/g)＝$2\ x_2 \times V_E \div W = 3\ x_2 \div W$

ADP (μg/g）=$2\ x_2 \times V_E \times 427.2 \div W = 1281.6\ x_2 \div W$

V_E: volume of extract solution I, 1.5mL; W: Sample weight, g; M_{ADP}: 427.2; 2: Sample dilution factor.

B. Liquid volume:

ADP (μmol/mL)＝$2\ x_2 \times V_E \div V_S = 5 \times x_2$

ADP (μg/mL）=$2\ x_2 \times V_E \times 427.2 \div V_S = 2136 \times x_2$

V_E: volume of extract solution I, 1mL; M_{ADP}: 427.2; V_S：volume of sample, 0.4mL；2: Sample dilution factor.

C. Cell amount

ADP (μmol/10^4 cell)＝$2\ x_2 \times V_E \div N = 0.002 \times x_2 \div N$

ADP (μg/g10^4 cell）=$2\ x_2 \times V_E \times 427.2 \div N = 854.4 \times x_2 \div N$

V_E: volume of extract solution I, 1mL; M_{ADP}: 427.2; N: number of cells, 10^4 as a unit; 2: Sample dilution factor.

IV. AMP content calculation

A. Sample weight

AMP (μmol/g)＝$2\ x_3 \times V_E \div W = 3\ x_3 \div W$

AMP (μg/g）=2 x_3 ×V_E×499.19÷W=1497.57x_3÷W

V_E: volume of extract solution I, 1.5mL; W: Sample weight, g; M_{AMP}: 499.19; 2: Sample dilution factor.

B. Liquid volume:

AMP (μmol/mL)＝2 x_3 ×V_E÷V_S=5×x_3

AMP (μg/mL）=2 x_3 ×V_E×499.19÷V_S =2495.95×x_3

V_E: volume of extract solution I, 1mL; M_{AMP}: 499.19; V_S： volume of sample, 0.4mL； 2: Sample dilution factor.

C. Cell amount

AMP (μmol/10^4 cell)＝2 x_3 ×V_E÷N =2×x_3÷N

AMP (μg/g10^4 cell）=2 x_3 ×V_E×499.19 ÷N =998.38×x_3÷N

V_E: volume of extract solution I, 1mL; M_{AMP}: 499.19; N: number of cells, 10^4 as a unit; 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 nucleotides in the sample. The peak area of nucleotides 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 nucleotides concentration in the sample is too high, it is recommended to dilute it before testing.
3. 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.