ATP、ADP、AMP Content HPLC Assay Kit

Note: Choose 2-3 sample with large expected differences for prediction before formal determination.

Detection Equipment: High performance liquid chromatography(HPLC)

Catalog Number : BC5114

Size: 50T/48S

Components:

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

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

Reagent I: Liquid 15 mL ×1 , Storage at 2-8℃. Before use, 3.5 mL of reagent I was added to 1000 mL of ultrapure water, and its pH was adjusted to 6.15 with reagent II to form mobile phase B and sealed.

Reagent II: Liquid 10 mL ×1 , Storage at 2-8℃.

ATP standard: Powder ×1, Storage at -20℃. The standard solution of 1 μmol/mL ATP was prepared by adding 1.8 mL distilled water and Storage at -20℃. To ensure the integrity of ATP, avoid repeated freezing and thawing.

ADP standard: Powder ×1, Storage at -20℃. ADP standard solution (1 μmol/mL) was prepared by adding 2.34 mL distilled water and Storage at -20℃. To ensure the integrity of ADP, avoid repeated freezing and thawing.

AMP standard: Powder ×1, Storage at -20℃. AMP standard solution of 1 μmol/mL was prepared by adding 2.0 mL distilled water and Storage at -20℃. To ensure the integrity of AMP, avoid repeated freezing and thawing.

Description:

Nucleotides have important biological functions. They are a group of compounds composed of purine or pyrimidine bases, ribose or deoxyribose, and phosphate, which are mainly involved in the formation of nucleosides.

Adenosine triphosphate (ATP) is considered to be a universal energy source essential for cellular synthesis for survival and reproduction in all organisms. ATP can be produced by a variety of cellular pathways. "Most typically, they are synthesized by adenosine triphosphate synthase through oxidative phosphorylation in mitochondria, or by photosynthesis in the chloroplasts of plants." 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, the product of the hydrolysis of adenosine triphosphate (ATP), which loses a phosphate group, breaks a high-energy phosphate bond, and releases energy."

Adenosine monophosphate (AMP) is widely found in animals, plants, microorganisms and cultured cells. It is formed after the release of energy from ATP and ADP 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 and AMP have absorption peaks at 254 nm, which can be used to determine different nucleotide contents according to different peak time and peak area by HPLC.

Reagents and Equipment Required but Not Provided:

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

Preparation before The Experiment:

1. 500 mL chromatographic pure acetonitrile (mobile phase A) and 1000 mL prepared mobile phase B were filtered by filter membrane to remove impurities in the solvent to prevent blocking the chromatographic column. (Acetonitrile was filtered by 0.45 μm organic filter membrane, and the prepared mobile phase B was filtered by 0.22 μm aqueous filter membrane).
2. The prepared mobile phases A and B were sonicated for 30 min to remove the gas in the solvent and prevent blocking the chromatographic column and affecting the experimental results.
3. Preparation of ATP standard: 1 μmol/mL ATP standard solution was diluted into 0.5 μmol/mL, 0.1 μmol/mL, 0.05 μmol/mL, 0.01 μmol/mL, 0.005 μmol/mL ATP standard solution with distilled water.
4. Preparation of ADP standard: 1 μmol/mL ADP standard solution was diluted into 0.5 μmol/mL, 0.1 μmol/mL, 0.05 μmol/mL, 0.01 μmol/mL, 0.005 μmol/mL ADP standard solution with distilled water. (The concentration of the prepared standard is for reference only and can be adjusted according to the actual sample concentration).
5. Preparation of AMP standard: 1 μmol/mL AMP standard solution was diluted into 0.5 μmol/mL, 0.1 μmol/mL, 0.05 μmol/mL, 0.01 μmol/mL, 0.005 μmol/mL AMP standard solution with distilled water. (The concentration of the prepared standard is for reference only and can be adjusted according to the actual sample concentration).
6. Filter into the brown injection bottle with water needle type filter to be tested (please place it in room temperature before testing, so as not to affect the retention time).

Protocol:

I. Sample Extraction

1. **Tissue sample:** According to the ratio of tissue mass (g) : extract solution to volume (mL) 1:5~10 (it is recommended to weigh 0.3 g tissue sample and add 1.5 mL extract solution), add extract solution 1, ice bath homogenate, and then soak in ice bath for 40 min. Centrifuge at 10000 rpm for 10 min at 4℃, take 750 μL of supernatant, add 750 μL of extract solution II, fully shake (5 min) and mix well, and then centrifuge at 10000 rpm for 10 min at 4℃ again. Take the supernatant and filter it through the water needle filter into the brown sample bottle to be measured at room temperature (within 2 hours).
2. **Cell sample:** According to the ratio of 10 million (pieces) : extract solution to volume (mL) 1000-500:1 (it is recommended to take 10 million cell sample and add 1 mL extract solution 1), add extract solution 1, ice bath ultrasonic crushing cells (power 300W, ultrasound 3 seconds, interval 7 seconds, total time 3 min); Centrifuge at 10000 rpm at 4℃ for 10 min, take 0.75 mL supernatant, add 0.75 mL extract solution II, fully shake (5 min) and mix well, and centrifuge at

10000 rpm at 4℃ for 10 min again. Take the supernatant and filter it through the water needle filter into the brown sample bottle to be measured at room temperature (within 2 hours).

3. **Serum:** It is recommended to weigh 0.4 mL serum sample, add 0.6 mL extract solution 1, and soak in ice bath for 40 min. Centrifuge at 10000 rpm for 10 min at 4℃, take 0.75 mL supernatant, add 0.75 mL extract solution II, fully shake (5 min) and mix well, then centrifuge at 10000 rpm again for 10 min at 4℃ to take the supernatant. Use a water needle filter to filter into the brown sample bottle inside room temperature to be measured (within 2 h).

II. Measurement Steps

1. Turn on the computer, turn on the switch button of each module of the liquid chromatograph, install the chromatographic column, and open the software. In the method group, set the injection volume as 10 μL, the column temperature as 27℃, the flow rate as 0.8 mL/min, and the wavelength as 254 nm.
2. The column was cleaned by mobile phase, and the column was equilibrated by mobile phase with acetonitrile: mobile phase B (pH= 6.15) = 2:98. After the baseline was stable, the sample was injected.
3. The injection volume of the prepared standard solution was 10 μL. ATP, ADP and AMP could be separated within 10 min. The retention time of ATP, ADP and AMP was about 7.8 min, 6.7 min and 5.4 min, respectively. (The retention time is different due to different systems, columns, mobile phase pH, etc., which is only used as a reference).
4. The injection volume of the prepared sample solution was 10 μL, and the peak areas of ATP, ADP and AMP were detected at the corresponding retention time.

Time	Mobile phase	
	Solvent A	Solvent 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

1. Establishment of standard curve

The standard curves of ATP, ADP and AMP were drawn with the concentration of standard (μmol/mL) as the abscordinate and the peak area as the ordinate. The peak area of the sample was substituted into the standard curve, and the concentrations of ATP, ADP and AMP in the sample x_1、x_2、x_3 (μmol/mL) were calculated.

2. Calculation of ATP content:

1) Calculated by sample quality:

The amount of ATP (μmol/g) = $2\ x_1 \times V_{extraction} \div W = 3 \times x_1 \div W$

$V_{extraction}$: Add the volume of Extract I, 1.5 mL; W: sample quality, g; 2: sample dilution.

2) Calculated by sample volume:

The amount of ATP (μmol/mL) = 2 x_1×$V_{extraction}$ ÷V_{sample} =5×x_1

$V_{extraction}$: total volume after adding Extract I, 1 mL; V_{sample}: add sample volume to Extract I, 0.4 mL; 2: sample dilution.

3) Calculated by cell number:

The amount of ATP (μmol/10^4 cells) =2 x_1 ×$V_{extraction}$ ÷N=2×x_1÷N

$V_{extraction}$: volume of Extract I, 1 mL; N: number of cells, in tens of thousands; 2: sample dilution.

3. Calculation of ADP content:

1) Calculated by sample quality:

The content of ADP (μmol/g) = 2x_2 ×$V_{extraction}$ ÷W=3×x_2÷W

$V_{extraction}$: Add the volume of Extract I, 1.5 mL; W: sample quality, g; 2: sample dilution.

2) Calculaed by sample volume:

The amount of ADP (μmol/mL) = 2x_2 ×$V_{extraction}$ ÷V_{sample} =5×x_2

$V_{extraction}$: Total volume after adding Extract I, 1 mL; V_{sample}: add sample volume to Extract I, 0.4 mL; 2: sample dilution.

3) Calculated by cell number:

The amount of ADP (μmol/104 cells) = 2x_2 ×$V_{extraction}$ ÷N=2×x_2 ÷N

$V_{extraction}$: Volume of Extract I, 1 mL; N: number of cells, in tens of thousands; 2: sample dilution.

4. Calculation of AMP content:

1) Calculated by sample quality:

AMP content (μmol/g) = 2x_3 ×$V_{extraction}$ ÷W=3×x_3÷W

$V_{extraction}$: Add the volume of Extract I, 1.5 mL; W: Sample quality, g; 2: sample dilution ratio.

2) Calculated by sample volume:

AMP content (μmol/mL) = 2x_3 ×$V_{extraction}$ ÷V_{sample} =5×x_3

$V_{extraction}$: Total volume after adding Extract I, 1 mL; V_{sample}: Add the sample volume to Extract I, 0.4mL; 2: sample dilution ratio.

3) Calculated by the number of cells:

AMP content (μmol/104 cell) = 2x_3 ×$V_{extraction}$ ÷N=2×x_3 ÷N

$V_{extraction}$: The volume of Extract I, 1 mL; Number of cells: tens of thousands, 10 million; 2: sample dilution ratio.

Note :

1. After the test is completed, it is necessary to flush the column with a high concentration of ultra-pure water (about 20-30 column volumes) to prevent blocking the column, and finally flush according to the type of column to prevent damage to the column.
2. The dilution ratio of the standard product is determined according to the concentration of different nucleotides in the sample, and the peak area of different nucleotides in the sample must be within the peak area of the corresponding standard solution of different concentrations, and the dilution ratio of the standard product is only a reference. If the concentration of one nucleotide in the sample is too high, it is recommended to dilute and then test.

It is recommended to use fresh sample for extraction. After extraction, ATP, ADP and AMP in the sample are not stable at room temperature and should be operated as soon as possible.