

Folic acid content Assay kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4834

Sizes: 50T/48S

Product Description:

Folic acid is a water-soluble vitamin, which is named for its rich content in green leaves, also known as tetrylglutamic acid. There are several forms in nature, and its parent compound is composed of three components: tretin, p-aminobenzoic acid and glutamic acid. Folic acid is one of the essential vitamins for the growth and reproduction of the body cells, the lack of folic acid will affect the normal physiological activities of the human body.

Folic acid has ultraviolet absorption at 210 nm, and its content can be determined by ultraviolet detector.

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, EP tube (1.5 mL), syringe filters (water), syringe, suction filter, filter membrane (organic, water), brown injection bottle, carbinol (chromatographically pure), ultrapure water.

Product Composition:

Extract solution: Liquid 30 mL×1. Storage at 2-8°C.

Reagent I: Liquid 5 mL×1. Storage at 2-8°C.

Reagent II: Liquid 1.5 mL×1. Storage at 2-8°C.

Reagent III: Powder×2. Storage at 2-8°C.

Standard: Powder×1. Store at 2-8°C. Before use, 1 mL 0.05 mol/L of KOH solution was added to prepare 5 mg/mL Folic acid standard solution, which was sealed and stored at 2-8°C, away from direct sunlight.

Preparations before the experiment:

1. Dissolve 1 bottle of Reagent III into 1000 mL of ultra-pure water, then add 0.55 mL of Reagent II and mix well to obtain mobile phase A.
2. Filter 1000 mL of prepared mobile phase A and methanol (chromatographically pure) with filter membrane. (The prepared mobile phase A was filtered by 0.22μm aqueous filter membrane, and methanol was filtered by 0.45μm organic filter membrane).
3. Ultrasound the filtered mobile phase for 20 min to remove bubbles.
4. Preparation of standard products: 5 mg/mL Pyridoxamine standard solution is diluted with distilled water into 500 μg/mL、100 μg/mL、20 μg/mL、4 μg/mL、0.8 μg/mL Folic acid standard solution. (The standard concentration is for reference only and can be adjusted according to the actual sample concentration). Store (sealed) at 4°C away from light, filter into brown sample bottle

with water needle filter before test, to be tested.

Procedure

I. Pyridoxamine extraction:

1. Tissue: According to the mass (g): Extract solution volume (mL) 1:5~10 ratio, it is recommended to weigh 0.1g sample (Fresh sample: chopped; Drying sample: Grinding and sifting) and add 0.6 mL Extract solution to fully homogenize), seal, mix evenly, and soak in a water bath at 60°C for 30 min. Cool to room temperature, add 0.1 mL of Reagent I, 0.3 mL distilled water, mix well, let stand for 2 min. Centrifuge at 10000 rpm for 10 min, take the supernatant (if there is still turbidity, it can be centrifuged again), filter it into the brown sample bottle using a water-based needle filter before the test, and then filter it again to be tested (if the color of the supernatant is too dark or the concentration is too high, it can be diluted and filtered again to be tested).

2. Cells: According to the number of cells (10⁴): the Extract solution volume (mL) is 10-50 million :1 ratio, it is recommended to take 50 million cells, add 0.6 mL of the Extraction solution, ultrasonic crushing cells (power 200W, ultrasonic 3s, intermittent 9s, repeat 30 times, total time 6 min), seal and mix well. Soak in a water bath at 60°C for 30 min. Cool to room temperature, add 0.1 mL of Reagent I, 0.3 mL of distilled water, mix well, leave for 2 min. Centrifuge at 10000 rpm for 10 min, take the supernatant (if there is still turbidity, it can be centrifuged again), and filter it into the brown sample bottle using a water-based needle filter before testing.

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: 30°C, flow rate 1 mL/min, and the ultraviolet detector wavelength to 210 nm. The sampling time of a single sample is 25 minutes, and the preservation method group is set.
2. Use the corresponding mobile phase to clean the column, balance the column with mobile phase A, and start adding samples after the baseline is stable.
3. Test the standard solution to be measured, the sample size is 10 μL, Folic acid can be separated within 25 min, and the retention time of Folic acid is about 20.5 min (the retention time is different with the system, column, mobile phase pH, temperature, etc., and is only for reference).
4. Test the sample solution to be measured, the injection volume is 10 μL, and test the peak area of Folic acid at the corresponding retention time.
5. Complete sequence sampling table: (including the cleaning and rebalancing process of the column after the determination of a single sample is completed)

Time (t)	Carbinol (%)	Mobile phase A (%)
0 min	0	100
1 min	0	100
1.1 min	3	97

8 min	3	97
8.1 min	15	85
23 min	40	60
25 min	40	60
25.1 min	60	40
35 min	60	40
35.1 min	0	100
45 min	0	100

III. Calculations:

The standard curve $y=kx+b$ was drawn with the standard concentration (ng/mL) as the horizontal coordinate x and the peak area as the vertical coordinate y . The peak area of the sample was substituted into the standard curve to calculate the concentration x (ng/mL) of Folic acid in the Extraction solution.

1. Tissue sample:

$$\text{Folic acid content } (\mu\text{g/g}) = x \times V_S \div W \times F = x \div W \times F$$

V_S : Sample population volume, 1 mL; W : Sample quality, g; F : dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio.

2. Cell sample:

$$\text{Folic acid content } (\mu\text{g} / 10^4 \text{cell}) = x \times V_S \div N \times F = x \div N \times F$$

V_S : Sample population volume, 1 mL; N : Cell number, 10^4 ; F : dilution ratio, the sample tested after dilution, the calculation needs to be multiplied by the corresponding dilution ratio.

Note:

Precautions:

- After the test is completed, it is necessary to flush the column with a high concentration of ultra-pure water phase (about 20-30 column volumes) to prevent blocking the column, and then flush the column with a high concentration of organic phase, and finally flush according to the type of column specification to prevent damage to the column.
- The dilution of the standard product should be determined according to the concentration of folic acid in the sample, and the peak area of folic acid in the sample must be within the peak area of the standard solution of different concentrations, and the dilution of the standard product is only a reference. If the folic acid concentration in the sample is too high, it is recommended to dilute it with distilled water and then test.
- If the sample size is too large, it is recommended to test the standard solution once a day (one concentration of the standard solution can be) to determine the corresponding retention time, and the solution to be tested must be placed at room temperature before testing.

4. In order to exclude the influence of gradient elution baseline drift, a blank detection can be performed.