Citric acid (CA) Content Assay Kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number: BC2154

Size: 50T/48S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution I	Liquid 80 mL×1	2-8℃
Extract solution II	Liquid 80 mL×1	2-8℃
Reagent I	Liquid 3 mL×1	2-8℃
Reagent II	Powder×2	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Reagent II: Dissolve one bottle of Reagent II into 1000 mL of ultrapure water, add 0.9 mL of Reagent I, mix well, and obtain mobile phase A.
2. 1000 mL of prepared mobile phase A was suctified with a membrane. (Prepared mobile phase A was suction with 0.22 μm aqueous membrane).
3. Ultrasonicate the filtered Mobile Phase A for 20 minutes to remove air bubbles.
4. Preparation of Standard: Before use, add 2 mL of distilled water to prepare a 10 mg/mL citric acid standard solution, and store it at 2-8℃ in a sealed container, avoiding direct sunlight. The 10 mg/mL citric acid standard solution was diluted with distilled water to 5000 μg/mL, 2500 μg/mL, 1250 μg/mL, 625 μg/mL, 62.5 μg/mL, and 31.25 μg/mL citric acid standard solutions, respectively (The prepared standard concentrations are for reference only and can be adjusted according to the actual sample concentration). Store (sealed) at 4°C in the dark, filter into the brown sample bottle with an aqueous syringe filter before testing, and wait for testing.

Product Description:

Citric acid is an important organic acid, also known as citrate. Due to its mild and refreshing sour taste, citric acid is widely used in the production of various beverages, wines, candies, snacks, biscuits, canned foods, dairy products, and other food items. In the market for all organic acids, citric acid holds a market share of over 70%, and there is currently no acidulant that can replace it.

Citric acid has an absorption peak at 210 nm, allowing its content to be determined using high-performance liquid chromatography.

Reagents and Equipment Required but Not Provided:

High-performance liquid chromatography (HPLC) instrument (with Polaris C18-A column (4.6×250 mm) and variable wavelength detector (VWD)), desk centrifuge, ultrasonic cleaner, adjustable pipette, mortar/homogenizer, EP tubes (2 mL), needle filters (for organic and aqueous solutions), syringes, filtration apparatus, filter membranes (for aqueous and organic solutions), 50 brown sample vials (1.5 mL), and ultrapure water.

Operation procedure

I. Extraction of citric acid:

1. **Plant samples:** According to the ratio of mass (g) to extraction solution volume (mL) of 1:5~10, it is recommended to weigh 0.15 g of fresh sample, grind it thoroughly, add 1 mL of Extract solution I, seal it, mix well, and place it in a 75℃ water bath for 20 minutes of extraction. Centrifuge at 12000 rpm for 10 minutes, take the supernatant, add 0.5 mL of Extract solution I to the filter residue again, shake to mix well, and place it in a 75℃ water bath for 20 minutes of extraction. Mix the two extractions, centrifuge at 12000 rpm for 10 minutes, take the supernatant, and filter it into a brown sample vial using an aqueous needle filter before testing (if the supernatant is too dark or concentrated, it can be diluted and filtered again before testing).
2. **Animal samples:** According to the ratio of mass (g) to extraction solution volume (mL) of 1:5~10, it is recommended to weigh 0.15 g of fresh sample, grind it thoroughly, add 1 mL of Extract solution II, seal it, mix well, and place it in a 75℃ water bath for 20 minutes of extraction. Centrifuge at 12000 rpm for 10 minutes, take the supernatant, add 0.5 mL of Extract solution II to the filter residue again, shake to mix well, and place it in a 75℃ water bath for 20 minutes of extraction. Mix the two extractions, centrifuge at 12000 rpm for 10 minutes, take the supernatant, and filter it into a brown sample vial using an organic needle filter before testing (if the supernatant is too dark or concentrated, it can be diluted and filtered again before testing).

II. Determination procedure:

1. Turn on the computer, switch on all modules of the liquid chromatograph, install the chromatographic column, open the software, set the injection volume to 10 μL in the method group, set the column temperature to 30℃, the flow rate to 0.4 mL/min, the wavelength to 210 nm, and the run time to 25 minutes. Save the method group after setting.
2. Clean the column with the corresponding mobile phase, balance the column with mobile phase A, and start adding samples after the baseline stabilizes.
3. Detect the standard solution to be tested with an injection volume of 10 μL. Citric acid can be separated within 25 minutes, and the retention time of citric acid is around 16.9 minutes (the retention time may vary depending on factors such as the system, column, pH of the mobile phase, and temperature, and is only provided as a reference).
4. Detect the sample solution to be tested with an injection volume of 10 μL, and measure the

peak area of citric acid at the corresponding retention time.

III. Calculation:

Plot a standard curve for citric acid with the concentration of the standard solution (μg/mL) as the abscissa and the peak area as the ordinate. Substitute the peak area of the sample into the standard curve to calculate the concentration x (μg/mL) of citric acid in the extraction solution.

The content of citric acid (μg/g mass) = x×V÷W=10x

V: The volume of the Extract solution, 1.5 mL;

W: Sample weight(g).

For samples tested after dilution, multiply by the corresponding dilution factor before calculation.

Note:

1. After the test is completed, the chromatographic column should be rinsed with a high concentration of ultrapure water phase (about 20-30 times the column volumes) to prevent blockage, followed by rinsing with a high concentration of organic phase. Finally, rinse the column according to its specific type to prevent damage.
2. The dilution factor of the standard solution should be determined based on the concentration of citric acid in the sample. The peak area of citric acid in the sample must within the range of peak areas obtained from standard solutions of different concentrations. The dilution factor for the standard solution is only a reference. If the concentration of citric acid in the sample is too high, it is recommended to dilute it before measurement.
3. If the sample volume is too large, it is advisable to test a standard solution (just one) once a day to confirm the corresponding retention time. All solutions to be tested should be brought to room temperature before testing.