Resveratrol Content Assay Kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4454

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	Liquid 100 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. 500 mL of methanol and 500 mL of ultrapure water were filtered with a membrane to remove impurities from the solvent to prevent column clogging. (0.45 μm organic membrane was used for methanol, and 0.22 μm aqueous membrane was used for ultrapure water).

2. Preparation of mobile phase: A: One bottle of 500 mL of ultrapure water after suction filtration. B: One bottle of 500 mL methanol after filtration. The prepared mobile phase was sonicated for 30 min to remove the gas in the solvent to prevent pressure instability and affect the experimental results.

3. Preparation of Standard: Before use, add 1 mL of extract solution to prepare a 1 mg/mL resveratrol standard solution. Sealing the mouth with parafilm after each use is recommended to prevent volatilization. 1 mg/mL resveratrol standard solution was diluted into 100 μg/mL, 50 μg/mL, 10 μg/mL, 5 μg/mL, and 1 μg/mL resveratrol standard solution with the extraction solution (the prepared standard concentration is for reference only and can be adjusted according to the actual sample concentration). It is filtered with a syringe filter and then tested. Sealing the mouth with parafilm after each use is recommended to prevent volatilization.

Product Description:

Resveratrol is a natural stilbene polyphenol substance, also known as pyrolicinol. Natural resveratrol has two configurations, trans and cis, and exists in plants such as peanuts, grapes, veratrol, knotweed, and mulberry, among which the content of knotweed, peanuts, and grapes is relatively abundant, and it mainly exists in trans.Resveratrol has a wide range of pharmacological effects and can be used as a raw material for healthy food and new drug development, especially anti-tumor drug development.

Resveratrol has an absorption peak at 306 nm, and its content can be determined by HPLC.

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Reagents and Equipment Required but Not Provided:

High Performance Liquid Chromatograph (C18 column (4.6×250 mm), ultraviolet detector (VWD)), benchtop centrifuge, ultrasonic cleaner, adjustable pipette, mortar/homogenizer, brown EP tube, syringe filter (50, organic, 0.45 μm), syringe, suction filter, membrane (one organic and one aqueous), brown sample vials (50, 2 mL), methanol (chromatographically grade, 500 mL), ultrapure water.

Operation procedure

I. Extraction of crude resveratrol:

The sample was placed in a dark place to dry, then put into a mortar and ground, weighed 0.15 g, and added to the brown EP tube, 1.5 mL of extract was added, protected from light, and leached overnight at room temperature. Then sonicate for 2 h (50 Hz, 40°C). Centrifuge at 10000 rpm for 10 minutes (25°C) and take the supernatant (if solids are still present in the solution, centrifuge again). The obtained supernatant was filtered through a syringe filter into a brown vial for testing.

II. Determination procedure:

1. Turn on the computer, turn on the switch buttons of each module of the liquid chromatograph, install the chromatographic column, open the software, set the injection volume to 10 μL, the column temperature: room temperature (about 25°C), the flow rate is 1 mL/min, the wavelength is 306 nm, the gradient elution procedure is as shown in the following table, the sampling time is 45 min, and the method set is saved after setting. (Includes column cleaning and equilibration after a single sample has been assayed).

Gradient time	Mobile phase	
	Phase A	Phase B
0 min	78%	22%
20 min	22%	78%
20.1 min	10%	90%
30 min	10%	90%
35 min	78%	22%
45 min	78%	22%

2. Clean the column with the mobile phase, equilibrate the column with the initial concentration ratio of the mobile phase, and start loading after the baseline is stable.

3. Detect the standard solution to be tested with an injection volume of 10 μL. Resveratrol can be separated within 20 minutes, and the retention time of resveratrol is around 13.6 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).Calculate the peak area of the resveratrol standard at different concentrations.

4. Add 10 μL of sample solution and measure the peak area of resveratrol at the corresponding

retention time.

III. Calculation:

Plot a standard curve for resveratrol 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 resveratrol in the extraction solution.

Content of resveratrol in the sample (μg/g) = x×V÷W=1.5x÷W

V: The volume of extract solution, 1.5 mL;

W: Sample weight(g).

Note:

1. The dilution factor of the standard is determined according to the concentration of resveratrol in the sample, and the peak area of resveratrol in the sample must be within the peak area of the resveratrol standard at different concentrations, and the dilution factor of this standard is only a reference.
2. After use, the column needs to be rinsed with a high concentration of organic phase and then rinsed according to the type of column specification to prevent damage to the column.
3. Resveratrol is unstable to light, and it is recommended that the standard solution and sample solution be used and stored in a dark environment.

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