Luteolin Content Assay Kit

Note: Before the experiment, it is recommended to select 2-3 sample with large expected differences for pre-experiment.

Operation Equipment: High performance liquid chromatography

Catalog Number: BC4524

Size: 50T/48S

Product Description:

Luteolin is a natural tetrahydroxyflavonoid compound found in various vegetables and fruits, with a wide range of pharmacological activities. It not only inhibits tumor cell proliferation, induces tumor cell apoptosis, and helps the body resist malignant tumors, but also has anti-inflammatory and antioxidant properties, protects the liver and cardiovascular system, and prevents and reduces osteoporosis.

Osmanthus extract has an absorption peak at 360 nm and its content can be determined by high-performance liquid chromatography.

Required reagents and equipment:

High performance liquid chromatography (C18 column (4.6 × 250 mm), UV detector (VWD)), desktop centrifuge, adjustable pipette, mortar/homogenizer, brown EP tube, needle filter (50, organic system, 0.45 μm), syringe, suction filter, filter membrane (organic system, water system), brown injection bottle (50, 1.5 mL), acetonitrile (chromatographically pure, 500 mL), ultrapure water, phosphoric acid (analytical pure), methanol (analytical pure), 5 mL white plastic reagent bottle, 2 mL EP tube.

Product Composition:

1. Extract solution I：Liquid 80 mL×1, stored at 2-8 °C.
2. Extract solution II：Liquid 90 mL×1, stored at 2-8 °C.
3. Standard：Powder×1, stored in the dark at 2-8 ℃. Before use, add 2 mL of methanol to prepare a 1 mg/mL standard solution of luteolin, seal and store at 2-8 ℃, and avoid direct sunlight.

Preparation before the experiment:

1. Add 1.18 mL of phosphoric acid to 500 mL of ultrapure water and mix thoroughly to obtain mobile phase A.
2. Filter 500 mL of chromatographically pure acetonitrile (mobile phase B) and 500 mL of prepared mobile phase A through a filter membrane to remove impurities and prevent column blockage. Acetonitrile was filtered using a 0.45 μm organic filter membrane, while the prepared mobile phase A was filtered using a 0.22 μm aqueous filter membrane.
3. Ultrasonic the prepared mobile phases A and B for 20 minutes each, and set aside for later use.
4. Preparation of standards: Dilute 1 mg/mL of luteolin standard solution with methanol to 0.1 mg/mL, 0.05 mg/mL, 0.01 mg/mL, 0.005 mg/mL, and 0.001 mg/mL of luteolin standard solution (the

concentration of the prepared standards is for reference only and can be adjusted according to the actual sample concentration). Store at 4℃ (sealed), filter with an organic needle filter into a brown injection bottle before testing.

Operation steps:

I. Extraction of luteolin:

1. Extraction of free luteolin:

Dry the tested plant samples in a 60 ℃ blast oven, grind them into powder, and sieve them through a 20-40 mesh sieve. According to the ratio of mass (g): volume of Extract solution I (mL) 1:10-20 (it is recommended to weigh 0.15 g of dried sample and add 1.5 mL of Extraction solution I), add it to a 2 mL EP tube, seal it, mix evenly, and then place it in an ultrasonic cleaning machine. Ultrasonic extraction should be carried out at room temperature (25-35 ℃, and attention should be paid to not exceeding the temperature during ultrasonic extraction) for 60 minutes. Centrifuge at 10000 rpm for 10 minutes at 4 ℃, take the supernatant (if there are still solid samples in the supernatant, centrifuge again), store at 4 ℃ (sealed), filter with an organic needle filter into a brown injection bottle before testing (if the supernatant color is too dark or the concentration is too high, dilute and filter again for testing).

2. Extraction of total luteolin:

Dry the tested plant samples in a 60 ℃ blast oven, grind them into powder, and sieve them through a 20-40 mesh sieve. According to the ratio of mass (g): volume of Extract solution II (mL) 1:10-20 (it is recommended to weigh 0.15 g of dried sample and add 1.5 mL of Extraction solution II), add it to the reagent bottle, weigh it, mix evenly, and then place it in an 80 ℃ constant temperature water bath for extraction for 2 hours (it is recommended to use a screw mouth 5 mL plastic reagent bottle and tighten it to prevent the bottle mouth from bursting during the heating process). After cooling, weigh again and add Extract solution II to make up for the difference. Sealed, placed in an ultrasonic cleaning machine, and subjected to ultrasonic extraction for 60 minutes at room temperature (25-35 ℃, with caution not to exceed the temperature during ultrasonic treatment). Then transfer the extracted turbidity to an EP tube and centrifuge at 10000 rpm for 10 minutes at 4 ℃. Take the supernatant (if there are still solid samples in the supernatant, centrifuge again) and store it at 4 ℃ (sealed). Before testing, filter it into a brown injection bottle using an organic needle filter and wait for testing (if the supernatant color is too dark or the concentration is too high, dilute it and filter it again for testing).

II. Measurement steps:

1. Turn on the computer, open the switch buttons of each module of the liquid chromatograph, install the chromatography column, open the software, set the injection volume to 10 μL, column temperature to 30 ℃, flow rate to 0.8 mL/min, wavelength to 360 nm, and single aliasing time to 30 minutes in the method group. After setting, save the method group.
2. Use the corresponding mobile phase to clean the column, and use a mobile phase equilibrium

column with a ratio of acetonitrile: mobile phase A=20:80. After the baseline is stable, start adding the sample.

3. Test the standard solution to be tested, with an injection volume of 10 μL. Lutein can be separated

within 30 minutes, and its retention time is about 14.3 minutes (the retention time varies depending on

the system, column, mobile phase pH, temperature, etc., and is only for reference).

4. Detect the sample solution to be tested, with an injection volume of 10 μL, and measure the peak area of luteolin at the corresponding retention time. (If continuous testing is required, the program elution can be performed according to the table below).

Time	Mobile phase	
	Mobile phase B	Mobile phase A
0 min	20%	80%
30 min	60%	40%
30.1 min	70%	30%
50 min	70%	30%
50.1 min	20%	80%
70 min	20%	80%

III. Calculation of Luteolin Content

Plot the standard curve of luteolin using the standard concentration (mg/mL) as the horizontal axis and peak area as the vertical axis. Substitute the peak area of the sample into the standard curve to calculate the concentration of luteolin in the sample x (mg/mL).

The content of luteolin (mg/g)=x × Ve ÷ W=1.5x ÷ W

Ve: volume of Extract solution, 1.5 mL;

W: Sample weight, g.

Note: The sample tested after dilution needs to be multiplied by the corresponding dilution factor before calculation.

Note:

1. After the test is completed, turn off the column temperature box heating system and wait for the column temperature to drop to room temperature or below before stopping the mobile phase.
2. After the test is completed, it is necessary to rinse the chromatography column with high concentration ultrapure water (about 20-30 column volumes) to prevent blockage of the chromatography column. Then, rinse the chromatography column with high concentration

organic phase, and finally rinse according to the specifications of the column type to prevent damage to the chromatography column.

3. The dilution factor of the standard should be determined based on the concentration of luteolin in the sample. The peak area of luteolin in the sample must be within the peak area of the standard solution at different concentrations. The dilution factor of the standard is only a reference. If the concentration of luteolin in the sample is too high, it is recommended to dilute it before testing.

4. If the sample size 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. All test solutions must be left at room temperature before testing.

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