# Shikimic Acid 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:** BC4734

**Size:** 50T/48S

**Product Description:**

Shikimic acid is present in the dry and mature fruit of the Magnoliaceae plant star anise.

Shikimic acid has an absorption peak at 210 nm and its content can be determined by high-performance liquid chromatography.

**Required reagents and equipment:**

High performance liquid chromatography (Polaris C18-A column (4.6 × 250 mm), UV detector (VWD)), desktop centrifuge, adjustable pipette, water bath, mortar/homogenizer, EP tube (2 mL), needle filter (aquatic systems), syringe, suction filter, filter membrane (organic system, water system), brown injection bottle (50, 1.5 mL), ultrapure water.

**Product Composition:**

1. Extract solution：Liquid 80 mL×1, stored at 2-8 °C.
2. Reagent I：Liquid 2 mL×1, stored at 2-8 °C.
3. Reagent II：Powder×2, stored at 2-8 °C.
4. Standard：Powder×1, stored in the dark at 2-8 ℃. Before use, add 2 mL of distilled water to prepare a 10 mg/mL standard solution of shikimic acid, seal and store at 2-8 ℃, and avoid direct sunlight.

**Preparation before the experiment:**

1. Dissolve a bottle of Reagent II in 1000 mL of ultrapure water, then add 0.9 mL of Reagent I and mix well to obtain mobile phase A.
2. Filter 1000 mL of prepared mobile phase A through a filter membrane. (The prepared mobile phase A is filtered using a 0.22 μm aqueous filter membrane.)
3. Filter the mobile phase A ultrasound for 20 minutes to remove any bubbles.
4. Preparation of standards: Dilute 10 mg/mL of shikimic acid standard solution with distilled water to 500 μg/mL、100 μg/mL、50 μg/mL、10 μg/mL、5 μg/mL、1 μg/mL、0.5 μg/mL of shikimic acid standard solution. (The concentration of the prepared standard sample is for reference only and can be adjusted according to the actual sample concentration.) Store in the dark at 2-8 ℃ (sealed), filter with a water-based needle filter into a brown injection bottle before testing.

**Operation steps:**

**I. Extraction of shikimic acid:**

According to the ratio of weight (g): volume of Extract solution (mL) 1:5-10, it is recommended to

weigh 0.15 g of sample, grind and crush it thoroughly, add 1 mL of Extract solution, seal it, mix evenly, and immerse it in a 75 ℃ water bath for 20 minutes. Centrifuge at 12000 rpm for 10 minutes, take the supernatant, add 0.5 mL of Extract solution to the filter residue, shake and mix well, and soak in a 75 ℃ water bath for 20 minutes. Mix the extracted liquids from two extractions, centrifuge at 12000 rpm for 10 minutes, take the supernatant, and filter it into a brown injection bottle using a water-based needle filter before testing (if the supernatant color is too dark or the concentration is too high, it can be diluted and filtered 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 0.4 mL/min, wavelength to 210 nm. The aliasing time is 25 minutes. After setting, save the method group.
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 tested, with an injection volume of 10 μL. Shikimic acid can be separated within 25 minutes, and its retention time is about 11.1 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 shikimic acid at the corresponding retention time.

**III. Calculation of shikimic acid content**

Plot the standard curve of shikimic acid using the standard concentration (μg/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 shikimic acid in the sample x (μg/mL).

The content of shikimic acid (μg/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, 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.
2. The dilution factor of the standard should be determined based on the concentration of shikimic acid in the sample. The peak area of shikimic acid 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 shikimic acid in the sample is too high, it is recommended to dilute it before testing.

3. 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.