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α-Ketoglutaric acid content detection kit

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

Operation Equipment: High performance liquid chromatography

Catalog Number:AK0027-50T-48S

Sizes： 50T/48S

Components:

Extract solution I: 80 mL×1. Storage at 4℃.

Extract solution II： 80 mL×1. Storage at 4℃.

Reagent I: 2mL×1. Storage at 4℃.

Reagent II: Powder×2. Storage at 4℃.

Standard： Powder×1. Storage at 4℃. Before use, 2 mL distilled water is added to prepare 10 mg / mL α-ketoglutaric acid standard solution, which is sealed at 4 °C to avoid direct sunlight.

Product Description：

α-Ketoglutaric acid（α-KG） is one of the two keto derivatives of glutaric acid. Its anion, α-ketoglutarate, is an important biological compound. It is a keto acid product of glutamic acid deamination and an intermediate product of the tricarboxylic acid cycle.

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

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, brown EP tube, syringe filters (water, organic), syringe, suction filter, filter membrane (organic, water), 50 brown injection bottle (1.5 mL), ultrapure water.

Preparations before the experiment:

1. One bottle of reagent II is dissolved in 1 000 mL ultrapure water, and then 0.9 mL of reagent 1 is added and mixed to obtain mobile phase A.

2. Filtration of 1000 mL mobile phase A with filter membrane. (The prepared mobile phase A is filtered by 0.22 μm aqueous membrane).

3. The filtered mobile phase A is sonicated for 20 min to remove bubbles.

4. The preparation of standard： 10 mg / mL of α-ketoglutarate standard solution is diluted with distilled water to 2500 μg / mL, 1250 μg / mL, 625 μg / mL, 62.5 μg / mL, 31.25 μg / mL, 6.25 μg / mL of α-ketoglutarate standard solution. (The prepared standard concentration is for reference only and can be adjusted according to the actual sample concentration). Stored in dark at 4℃ (sealed). The water system needle filter is used to filter into the brown injection bottle for testing.

Procedure

I. Sample preparation:

A． Plant:

According to the ratio of tissue (g): extract solution I (mL) = 1:5~10 (it is recommended to weigh 0.15 g tissue sample and add 1 mL extract solution I) to add extract solution I, homogenate on ice, and extract at 75℃ for 20 min. Centrifuge at 12000 rpm for 10 min, take supernatant; Add 0.5 mL of extract 1 to the filter residue again, shake and mix well, and soak in a 75℃ for 20 min. Mix the extract twice, centrifuged at 12000 rpm for 10 min, take the supernatant. Before the test, the water system needle filter is used to filter into the brown injection bottle for testing (if the supernatant is too dark or the concentration is too high, it could be diluted and filtered again).

B． Animal:

According to the ratio of tissue (g): extract solution I (mL) = 1:5~10 (it is recommended to weigh 0.15 g tissue sample and add 1 mL extract solution I) to add extract solution I, homogenate on ice, and extract at 75℃ for 20 min. Centrifuge at 12000 rpm for 10 min, take supernatant; Add 0.5 mL of extract 1 to the filter residue again, shake and mix well, and soak in a 75℃ for 20 min. Mix the extract twice, centrifuged at 12000 rpm for 10 min, take the supernatant. Before the test, the water system needle filter is used to filter into the brown injection bottle for testing (if the supernatant is too dark or the concentration is too high, it could be diluted and filtered again).

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 0.4 mL/min, and wavelength 210 nm, the elution program is as shown in the table below, and the sampling time is 25 min. After setting, save the method group.
2. Clean the column with the mobile phase, equilibrate the column with mobile phase A, and start the injection after the baseline is stable.
3. Detect the prepared standard solution, the injection volume is 10 μL, the α-KG can be separated within 25 minutes, and the retention time of α-KG is about 11.5 min (the pH of the system, column, mobile phase, etc. are different, the retention time is different, only reference).
4. Detect the prepared sample solution, the injection volume is 10 μL, and detect the peak area of α-KG at the corresponding retention time.

III. Calculations:

I. Draw a standard curve of α-KG with the standard concentration (μg/mL) as x and the peak area as the y. Substitute the peak area of the sample into the standard curve to calculate the α-KG concentration x (μg/mL) in the sample.

II. α-KG content caculation

α-KG content (μg/g)= x ×V_E÷W= 1.5x÷W

V_E: volume of extract solution I, 1.5 mL; W: Sample weight, g.

Note:

Precautions:

1. After the detection, the chromatographic column needs to be flushed with high-concentration ultrapure water (about 20-30 column volumes) to prevent clogging the chromatographic column. Finally, flush the column according to the specifications of the column to prevent damage to the chromatographic column.

2. The dilution factor of the standard should be determined according to the concentration of α-KG in the sample. The peak area of α-KG in the sample must be within the peak area of the standard solution of different concentrations. The dilution factor of the standard is only a reference. If the ATP concentration in the sample is too high, it is recommended to dilute it before testing.

4. If the sample number 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.