Phytoanthocyanins Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: NA0520

Size: 100T/48S

Components:

Extract solution: Liquid 50 mL ×1. Storage at 4℃.

Reagent I: Liquid 15 mL×1. Storage at 4℃.

Reagent II: Liquid 15 mL×1. Storage at 4℃.

Product Description

Anthocyanin is a kind of edible natural pigment which is easily soluble in water and other solvents. Anthocyanins give plants a colorful color. It also has a variety of health functions. Therefore, it has a broad application prospect in natural edible pigments, health products and pharmaceutical industry.

According to the structure and properties of anthocyanins at different pH, the content of anthocyanins can be determined. The maximum absorption peak of anthocyanin is found at 530 nm when the pH is 1. When the pH is 4.5, anthocyanins are converted to colorless chalcone and there is no absorption peak at 530 nm. The content of anthocyanin can be calculated by measuring the absorbance values at 530 nm and 700 nm at different pH.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/microplate reader, table centrifuge, water-bath, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/homogenizer and distilled water.

Procedure

I. Sample processing :

According to the tissue weight (g) : the volume of Extract solution (mL) is 1:5-10. (It is recommended that add 1 mL of Extract solution to 0.1 g tissue). Homogenate in ice bath, then transfer to EP tube. Dilute to 1 mL with the Extract solution. Cover tightly and extract at 60℃ for 30 min. Several shocks during the period. Centrifuge at 12000 rpm for 10 minutes at room temperature. Take the supernatant for test.

II. Determination Procedure

1. Preheat the spectrophotometer for more than 30 minutes and set the counter to zero with distilled water.
2. Operation table: (in 1.5 mL centrifuge tube/96 well flat-bottom plate)

Reagent Name (μL)	Test tube 1	Test tube 2
Sample	20	20
Reagent I	180	-
Reagent II	-	180

Mix thoroughly. Measure the absorbance of Test tube1 and Test tube2 at 530 nm and 700 nm, respectively. The absorbance values of Test tube1 at 530 nm and 700 nm are recorded as A1 and A1'. The absorbance values of Test tube2 at 530 nm and 700 nm are recorded as A2 and A2'. ΔA=(A1-A1')-(A2-A2').

III. Calculation of Anthocyanins:

1. Micro glass cuvette

1) Calculate by sample weight

Anthocyanins content (μmol/g fresh weight)=[ΔA÷(ε×d)×10^3×F]×V_E÷W=0.037×ΔA×F÷W

2) Calculate by Sample protein concentration

Anthocyanins content (μmol/mg prot)=[ΔA÷(ε×d)×10^3×F]×V_E÷(Cpr×V_E)=0.037×ΔA×F÷Cpr

F: Dilution Factor, 10;

d: Optical diameter of cuvette,1 cm;

W: Sample weight, g;

ε: Molar extinction coefficient of chromoside, 2.69×10^4 mL/mmol/cm;

V_E: Extract volume, 1 mL;

10^3：1mmol=10^3 μmol;

Cpr: Sample protein concentration, mg/mL；(The protein concentration needs to be extracted separately by PBS and then determined).

2. 96 well flat-bottom plate

1) Calculate by sample weight

Anthocyanins content (μmol/g fresh weight)=[ΔA÷(ε×d)×10^3×F]×V_E÷W=0.062×ΔA×F÷W

3) Calculate by Sample protein concentration

Anthocyanins content (μmol/mg prot)=[ΔA÷(ε×d)×10^3×F]×V_E÷(Cpr×V_E)=0.062×ΔA×F÷Cpr

F: Dilution Factor, 10;

d: Optical diameter of cuvette,0.6 cm;

W: Sample weight, g;

ε: Molar extinction coefficient of chromoside, 2.69×10^4 mL/mmol/cm;

V_E: Extract volume, 1 mL;

10^3：1mmol=10^3 μmol;

Cpr: Sample protein concentration, mg/mL；(The protein concentration needs to be extracted separately by PBS and then determined).

Note:

1. If A1 is greater than 1, the dilution ratio can be increased appropriately. Ensure that the total volume is 0.2 mL. Such as, add 10 μL of supernatant to 180 μL of Reagent I (equivalent to 20 times of dilution). If A1 is less than 0.1, the dilution ratio can be reduced appropriately. Ensure that the total volume is 1 mL. Such as, add 100 μL of supernatant to 100 μL of Reagent I (equivalent to 2 times of dilution). Keep A1 in the range of 0.1-1. It can improve the detection sensitivity. Note that the volume ratio of supernatant and the volume of Reagent Ⅱ should also be adjusted; when calculating, the actual dilution multiple should be

substituted into the following formula.

2. Because the Extract solution will denature the protein, if use the protein concentration to calculate, it needs to be extracted separately with PBS and then measured.

Examples:

1. Add 0.1g grape peel to 1mL extract solution and mix thoroughly, transfer to EP tube, seal with parafilm to avoid volatilization, immerse and extract at 60℃ for 30 min., several shocks during the period. Dilute to 1 mL with the Extract solution, centrifuge at 12000 rpm for 10 minutes at room temperature, take the supernatant, follow the determination procedure to operate, with 96-well flat-bottom plates to calculate: ΔA=(A1-A1')-(A2-A2')=(0.573-0.060)-(0.120-0.060)=0.453, according with mass of sample to calculate: Anthocyanins content (μmol/mg mass)=0.062×ΔA×F÷W =0.062×0.453×10÷0.1=2.81μmol/g mass.

Related Products:

NA0769/NA0528	Ceruloplasmin (CP) Assay Kit
NA0768/NA0527	Total antioxidant capacity (T-AOC) Assay Kit
NA0763/NA0522	Uric acid(UA)Assay Kit