Phytoanthocyanins Assay Kit

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

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: BC1385

Size: 100T/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	Storage
Extract Solution	Solution 50 mL×1	2-8℃
Reagent I	Solution 15 mL×1	2-8℃
Reagent II	Solution 15 mL×1	2-8℃

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 was 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 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. Cover tightly and extract at 60℃ for 30 min. Several shocks during the period. After extraction, dilute to 1mL with the Extraction solution. Centrifuge at 12000 rpm for 10 minutes at room temperature. Take the supernatant for test.

II. Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes and the spectrophotometer set the counter to zero with distilled water.
2. Operation table: (in 96 well 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 weight) = $[\Delta A \div (\varepsilon \times d) \times 10^3 \times F] \times V_E \div W = 0.037 \times \Delta A \times F \div W$

2) Calculate by Sample protein concentration

Anthocyanins content (μmol/mg prot) = $[\Delta A \div (\varepsilon \times d) \times 10^3 \times F] \times V_E \div (Cpr \times V_E) = 0.037 \times \Delta A \times F \div Cpr$

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 weight) = $[\Delta A \div (\varepsilon \times d) \times 10^3 \times F] \times V_E \div W = 0.062 \times \Delta A \times F \div W$

3) Calculate by Sample protein concentration

Anthocyanins content (μmol/mg prot) = $[\Delta A \div (\varepsilon \times d) \times 10^3 \times F] \times V_E \div (Cpr \times V_E) = 0.062 \times \Delta A \times F \div Cpr$

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 190 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 ReagentII 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 and take the supernatant, follow the determination procedure to operate, with 96-well plate to calculate: ΔA = (A1-A1') - (A2-A2') = (0.573-0.060) - (0.120-0.060) = 0.453, according with weight of sample to calculate: Anthocyanins content (μmol/mg weight)=0.062×ΔA×F÷W =0.062×0.453×10÷0.1=2.81μmol/g weight.

Recent Product citations:

[1] Hu H, Zhou XY, Wang YS, Zhang YX, Zhou WH, Zhang L. Effects of particle size on the structure, cooking quality and anthocyanin diffusion of purple sweet potato noodles. Food Chem X. 2023 Apr 3;18:100672. doi: 10.1016/j.fochx.2023.100672. PMID: 37091512; PMCID: PMC10114142.

[2] Shan C, Luo Y, Yang C, Gao X. The Effects of Poly-纬-Glutamic Acid on the Postharvest Physiology and Quality of Strawberry cv. Hongyan during Cold Storage. Foods. 2023 Aug 3;12(15):2944. doi: 10.3390/foods12152944. PMID: 37569213; PMCID: PMC10419068.

[3] Sylvia C, Sun J, Zhang Y, Ntini C, Ogutu C, Zhao Y, Han Y. Genome-Wide Analysis of ATP Binding Cassette (ABC) Transporters in Peach (Prunus persica) and Identification of a Gene PpABCC1 Involved in Anthocyanin Accumulation. Int J Mol Sci. 2023 Jan 18;24(3):1931. doi: 10.3390/ijms24031931. PMID: 36768256; PMCID: PMC9916050.

[4] Dai Y, Zhang L, Sun X, Li F, Zhang S, Zhang H, Li G, Fang Z, Sun R, Hou X, Zhang S. Transcriptome analysis reveals anthocyanin regulation in Chinese cabbage (Brassica rapa L.) at low temperatures. Sci Rep. 2022 Apr 15;12(1):6308. doi: 10.1038/s41598-022-10106-1. PMID: 35428824; PMCID: PMC9012755.

[5] Zhang J, Zhao J, Tan Q, Qiu X, Mei S. Comparative transcriptome analysis reveals key genes associated with pigmentation in radish (Raphanus sativus L.) skin and flesh. Sci Rep. 2021 Jun 1;11(1):11434. doi: 10.1038/s41598-021-90633-5. PMID: 34075070; PMCID: PMC8169917.

Related Products:

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BC1310/BC1315	Total antioxidant capacity (T-AOC) Assay Kit
BC1360/BC1365	Uric acid (UA)Assay Kit