Tannin content Assay Kit

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

Operation Equipment: Spectrophotometer

Catalog Number: BC1390

Size:50T/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 75 mL×2	2-8℃
Reagent I	Powder×1	RT
Standard	Powder×1	2-8℃

Solution preparation:

Standard : 10 mg tannic acid. Add 1.175 mL of Extract solution to dissolve it into 5000 nmol/mL standard solution before use. It could be stored at 2-8℃ for two weeks.

Product Description

Tannins are also called plant polyphenols. It is a kind of polyphenol compound widely existing in plants. Tannins can be used as potential biomarkers. The ability to bind to proteins is also known as convergence or astringency. Its convergence is the basis of many physiological activities, such as hemostasis, anti-tumor, anti-aging and other physiological activities. It is also one of the factors that affect the taste of the product.

According to the spectral characteristics, tannins have strong UV absorption at 275 nm. Activated carbon can adsorb tannin specifically. The tannin content can be detected by this property.

Reagents and Equipment Required but Not Provided.

Ultraviolet spectrophotometer, 1 mL quartz cuvette, centrifuge, water bath, adjustable pipette, 30-50 mesh screen, distilled water.

Procedure

I. Sample processing:

Dry the sample to constant weight and crush it. Over 30-50 mesh screen. Add 2 mL of Extract solution to 0.1 g sample weight. Sealing film to prevent liquid splashing. Extraction in 70℃ water bath for 30 min. Continuous oscillation. Centrifuge at 12000 rpm for 10 min at 25℃. Take the supernatant, and use the Extract solution to volume the supernatant to 2 mL for test.

II. Determination Procedure

1. Preheat the spectrophotometer/microplate reader for more than 30 minutes, adjust the wavelength to 275

nm and set the counter to zero with extract solution.

2. Standard working solution: diluted the 5000 nmol/mL standard solution to 25、12.5、6.25、3.125、1.5625、 0.78125 nmol/mL standard solution with the extraction solution.

3. The dilution of standard solution can refer to the following table:

Number	Pre dilution concentration (mg/mL)	Standard liquid volume (μL)	Volume of standard dilution solution (μL)	Diluted concentration (mg/mL)
1	5000	100	900	500
2	500	200	3800	25
3	25	2000	2000	12.5
4	12.5	2000	2000	6.25
5	6.25	2000	2000	3.125
6	3.125	2000	2000	1.5625
7	1.5625	2000	2000	0.78125

4. Operation table:

Reagent	Test tube (A_T)	Control tube(A_C)	Standard tube (A_S)	Blank tube (A_B)
Reagent I (mg)	about 10-15 mg	-	-	about 10-15 mg
Extract solution (mL)	-	-	-	1
Standard (mL)	-	-	1	-
Sample (mL)	1	1	-	-
Mix thoroughly and shock for 5 min. Centrifuge at 13000 g for 20 min (If there are still particles or turbidity in the supernatant, please centrifuge repeatedly until completely clear). Take supernatant to determine the absorbance at 275 nm. Record as A_C, A_T, A_S, A_B, and calculate $\triangle A_S = A_S - A_B$, $\triangle A_T = A_T - A_C$. Each test tube should be provided with one contrast tube. Standard curve and blank tube only need to be measured once or twice.				

III. Calculation of Tannin content:

1. Standard curve

Taking the concentration of each standard solution as the y-axis and its corresponding ΔA_S as the x-axis, draw a standard curve to get the standard equation y = kx + b, and bring ΔA_T into the equation to get y (nmol/mL).

2. Calculate

1) Calculate by protein concentration

Tannin content (nmol/mg prot)=$y \times V_E \div (V_E \times Cpr) = y \div Cpr$

2) Calculate by sample weight

Tannin content (nmol/g weight)=$y \times V_E \div W = 2y \div W$

V_E: Extract solution volume, 2 mL;

W: Sample weight, g;

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

Note:

1. If the absorbance value exceeds the linear range, the sample size can be increased or diluted before proceeding with the measurement. Pay attention to synchronously modifying the calculation formula.
2. Extraction contains ingredients that denature proteins, and protein content needs additional measurement if tannin content would be calculated by protein concentration.

Related Products:

BC1300/BC1305	Ceruloplasmin (CP) Assay Kit
BC1310/BC1315	Total antioxidant capacity (T-AOC) Assay Kit
BC1360/BC1365	Uric acid（UA）Assay Kit

Tel: 86-010-50973105 https://www.solarbio.net E-mail: info@solarbio.com