

## Tannin content Assay Kit

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

**Operation Equipment:** Spectrophotometer

**Catalog Number:** AK0440

**Size:**50T/48S

### Components:

Extract solution: Liquid 75 mL ×2. Storage at 4°C .

Solution I: Powder ×1. Storage at room temperature.

Standard: Powder ×1, 10 mg of tannic acid, store at 4°C . Add 1.175 mL of extraction solution to dissolve it into 5000 nmol/mL standard solution before use.

### 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 astringency 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, centrifuge, water bath, adjustable pipette, 1 mL quartz cuvette and distilled water.

### Procedure

#### I. Sample processing:

Dry the sample to constant weight and crush it. Over 40 mesh screen. Add 2 mL of Extract solution to 0.1 g sample weight. Sealing film to prevent liquid splashing. Extraction in 70°C water bath for 30 min. Continuous oscillation. Centrifuge at 12000 rpm for 10 min at 25°C . Take the supernatant. 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 distilled water.
2. 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. Sampling table:

Reagent Name (μL)	Control tube (A <sub>C</sub> )	Test tube (A <sub>T</sub> )	Standard tube (A <sub>S</sub> )	Blank tube (A <sub>B</sub> )
Reagent I (mg)	about 10- 15 mg	-	-	about 10- 15 mg

Distilled water (mL)	-	-	-	1
Standard (mL)	-	-	1	-
Sample (mL)	1	1	-	-

Mix thoroughly. Shock 5 min. Centrifuge at 15000 g for 20 min (If there are still particles or turbidity in the supernatant, please centrifuge repeatedly until completely clear). Take 200  $\mu$ L of supernatant to determine the absorbance at 275 nm. Record as  $A_C$ ,  $A_T$ ,  $A_S$ ,  $A_B$ .  $\Delta A_T = A_C - A_T$ .  $\Delta A_S = A_S - A_B$ .

### III. Calculation of Pyruvate content:

#### 1. Standard curve

Take the concentration of each standard solution as x-axis, and the corresponding  $\Delta A$  standard is y-axis. Then the linear regression equation  $y = kx + b$  is obtained. Bring  $\Delta A$  into the equation to get x (nmol/mL).

#### 2. Calculate

##### 1) Calculate by protein concentration

Tannin content (nmol/mg prot) =  $x \times V_E \div (V_E \times C_{pr}) = x \div C_{pr}$

##### 2) Calculate by sample weight

Tannin content (nmol/g fresh weight) =  $x \times V_E \div W = x \div W$

$V_E$ : Extract solution volume, 4 mL;

$C_{pr}$ : Sample protein concentration, mg/mL; (The protein concentration needs to be re extracted by PBS and then determined.)

$W$ : Sample weight, g.

1000:1  $\mu$ mol=1000 nmol

#### Note:

If the absorbance value determined by the sample is beyond the standard curve range, the sample should be diluted or concentrated properly before determination.

#### Related Products:

AK0254/AK0253	Ceruloplasmin(CP) Assay Kit
AK0456/AK0455	Total antioxidant capacity(T-AOC) Assay Kit
AK0446/AK0445	Uric acid (UA) Assay Kit