Total plant phenol detection kit

(Cat/No.:BC162 Size:50T/24S)

1. Determination principle

Under alkaline conditions, phenolics reduce tungsten molybdic acid to produce blue compounds with characteristic absorption peak at 760nm and total phenolic content at 760nm by measuring the light absorption value at 760 nm.

2. Instrument and Equipment (self-provided)

Oven, pulverizer, 40 mesh screen, ultrasonic breaker, mortar, centrifuge, visible spectrophotometer, 1 mL glass cuvette

3. Composition:(The kit is vaild for 3 months)

Extract: 60% ethanol aqueous solution, self-prepared;

Reagent 1: yellow liquid 8 mL 1 bottle, 4℃ storage;

Reagent 2:15 mL, 1 bottle of colorless liquid, 4℃ for storage;

Reagent 3:1mL 1 bottle of 2000 μ mol / L standard, colorless liquid, 4℃; 500 μmol/ L standard configuration: take 2000 μ mol / L standard at 1:3 (V: V): 3 times volume of 60% ethanol solution, as available.

4. Operating steps

1) Total phenol extraction:

Dry sample extraction: the sample was dried to constant weight, crushed. After 40 mesh sieve, weighed about 0.1g, add 2mL extract, extracted by ultrasonic extraction method, ultrasonic power was 300W, broken for 5 seconds, intermittent for 8 seconds, and 60℃ for 30min. After centrifugation at 4000 rpm / min for 10min, the supernatant was
removed for testing.

Fresh sample extraction: Put the fresh or frozen plant samples, ground with liquid nitrogen, weigh about 0.1g of plant powder in a covered centrifuge tube, add 2mL extract, cover tightly, vortex and extract for 2~3 minutes, put at 60℃ for 30min, 4000 rpm for 10min, and take the supernatant for testing.

Note: The supernatant requires a sample supernatant and a widely different sample to determine the optimal sampling concentration (tube OD-tube OD≤0.9).

2) Determination and operation table:

	Blank tube	standard tube	Determined tube	Control tube
60% aqueous ethanol solution (μ L)	50			
500 μmol/L Standard (μ L)		50		
Sample supernatant (μ L)			50	50
Reagent 1 (μ L)	250	250	250	
Mix well and stand at room temperature for 2min				
Reagent 2 (μ L)	250	250	250	250
distilled water (μL)	450	450	450	700

Mix well, let at room temperature for 10 min, distilled water to zero, wavelength 760nm, 1 mL cuvette, determine the absorbance value A of each tube .

Note: Control tubes need to be made for each sample to minimise errors.

5. Computational formula

$$\text{Total plant phenol content (organisations } \mu\text{mol/g)} = \frac{A_{measured} - A_{control}}{A_{standard} - A_{blank}} \times C_{standard} \times V_{sample\ total} \times N \div W$$

$C_{standard}$: Standard concentration, 500 μmol/L;

$V_{Sample\ total}$: Total volume of the supernatant, 0.002L;

N: dilution of supernatant;

W: Sample quality, g.

6. Calculation examples

After taking the leaves of frozen plants and taking the extraction of fresh samples, the supernatant is diluted by 3 times and the operation according to the experimental steps, the OD value of the blank tube is 0.001, the OD value of the standard tube is 0.502, the OD value of the measured tube is 0.461, and the OD value of the control tube is 0.000, then the results are:

$$\text{Total plant phenol content (organisations } \mu\text{mol/g)} = \frac{0.461 - 0.000}{0.502 - 0.000} \times 500 \times 0.002 \times 3 \div 0.102$$

$$= organisations 27.06 \mu\text{mol/g}$$

7. Determination significance

Plant phenolics have the effect of scavenging free radicals, antioxidant and anti-aging, have high nutritional value and medical care effect, and are widely used in cosmetics, food, medicine and other fields

8. Notes

9.

1) the reagent has a certain irritation of skin, please take protective measures when operation.
2) This experiment can make standard curve calculation by itself, or can be calculated according to the operation table according to the calculation formula, without affecting the results.
3) Samples can be dried or used with fresh samples according to the customer's own requirements. When the result unit is expressed by μ g / g tissue, it should be multiplied by the corresponding average molecular weight of total plant phenol.
4) At room temperature 25℃, can be directly reacted under room conditions.
5) Try to avoid strong light exposure during the reaction.
6) This kit is used only for scientific research.

Appendix I Preparation of standard curves for total plant phenol

1. Pre-experiment preparation:

2000 μmol/L (300 mg/L) standard was diluted in 60% ethanol into 1000 μmol/L, 500 μmol/L, 250 μmol/L and 125 μmol/L.

2. Operating steps:

	Blank tube	standard tube

60% aqueous ethanol solution (μ L)	50	
Different concentrations of standard (μ L)		50
Reagent 1 (μ L)	250	250
Mix well and stand at room temperature for 2min		
Reagent 2 (μ L)	250	250
distilled water (μL)	450	450
Mix well, let it stand at room temperature for 10 min, adjust the distilled water to zero, with the wavelength of 760nm, 1 mL cuvette, and determine the absorbance value of each tube.		

3. Experimental data:

Standard concentration (μmol/L)	0	125	250	500	1000
Standard OD values	0.006	0.135	0.251	0.462	0.924
Absolute OD value	0	0.129	0.245	0.456	0.918

4. The drawing is as follow