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CheKine™ Micro Plant Oligomeric Proantho Cyanidins (OPC) Assay Kit

Cat #: KTB1520 Size: 48 T/96 T

	Micro Plant Oligomeric Proantho Cyanidins (OPC) Assay Kit		
REF	**Cat #:** KTB1520	LOT	**Lot #:** Refer to product label
	Detection range: 0.39-50 mg/g		**Sensitivity:** 0.39 mg/g
	Applicable samples: Plant Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Oligomeric Proantho Cyanidins (OPC) are a class of polyphenolic compounds of flavanol monomers and their polymers. They are widely present in various organs of plants. They have strong antioxidant and free radical scavenging effects. Used in medicine, food, cosmetics, health care products industry. CheKine™ Micro Plant Oligomeric Proantho Cyanidins (OPC) Assay Kit provides a simple method for detecting OPC concentration in a variety of biological samples such as plant tissues. Under acidic conditions, the resorcinol and phloroglucinol on the A ring of plant proanthocyanidins undergo condensation reaction with vanillin to produce colored compounds. There is a characteristic absorption peak at 500 nm. The light absorption value at 500 nm can be measured to calculate the procyanidins content. The kit is used for testing plant samples.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Hydrochloric Acid	5 mL	10 mL	4℃
Vanillin	1	1	4℃, protected from light
OPC Standard	1 (5 mg)	1 (10 mg)	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 500 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, ice maker, 40 mesh sieve
- Deionized water, methanol
- Homogenizer

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Hydrochloric Acid: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Vanillin: Before use, add 5 mL methanol for 48 T, add 10 mL methanol for 96 T. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Note: The prepared Vanillin should be used as soon as possible, and stored at 4℃ up to one month.

Work Reagent: Before use, mix hydrochloric Acid and dissolved Vanillin at a ratio of 1:1. Equilibrate to room temperature before use. Store at 4℃, protected from light.

OPC Standard: Before use, add 0.5 mL Extraction Buffer for 48 T, add 1 mL Extraction Buffer for 96 T. The concentration is 10 mg/mL. Equilibrate to room temperature before use. Store at 4℃, protected from light.

Setting of standard curves: Further dilute the 10 mg/mL Standard to 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.078, 0.039 mg/mL standard solution with deionized water, as shown in the following table.

Num.	Volume of OPC Standard (μL)	Volume of Deionized Water (μL)	Standard Concentration (mg/mL)
Std.1	100 μL 10 mg/mL	0	5
Std.2	100 μL of Std.1 (5 mg/mL)	100	2.5
Std.3	100 μL of Std.2 (2.5 mg/mL)	100	1.25
Std.4	100 μL of Std.3 (1.25 mg/mL)	100	0.625
Std.5	100 μL of Std.4 (0.625 mg/mL)	100	0.313
Std.6	100 μL of Std.5 (0.313 mg/mL)	100	0.156
Std.7	100 μL of Std.5 (0.156 mg/mL)	100	0.078
Std.8	100 μL of Std.5 (0.078 mg/mL)	100	0.039

Note: Always prepare fresh standards per use; Diluted standard solution is unstable and should not be stored for a long time.

Sample Preparation

1. Plant tissues with more fibers: the plant tissue can be dried to constant weight, pulverized and sieved by a 40-mesh sieve. Weigh about 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 30 min (power 30% or 300 W, ultrasonic 5 s, interval 8 s). Centrifuge at 12,000 rpm for 10 min at 25℃. Take the supernatant, and dilute the volume to 1 mL with Extraction Buffer, and place it on ice to be tested.

2. Plant tissues with less fibers: Weigh about 0.1 g tissue, add 1 mL Extraction Buffer and mash. Ultrasonic break in ice bath 30 min (power 30% or 300 W, ultrasonic 5 s, interval 8 s). Centrifuge at 12,000 rpm for 10 min at 25℃. Take the supernatant, and dilute the volume to 1 mL with Extraction Buffer, and place it on ice to be tested.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 500 nm, visible spectrophotometer was returned to zero with deionized water.

2. Sample measurement (The following operations are operated in the 96-well plate or microglass cuvette).

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)	Control well (μL)

Sample	0	0	40	40
Different Concentration of Std.	0	40	0	0
Deionized Water	40	0	0	160
Work Reagent	160	160	160	0

3. Mix well and kept at 30℃ for 30 min. The absorbance value is measured at 500 nm. The blank well is marked as A_{Blank}, the standard well is marked as $A_{Standard}$, the test well is marked as A_{Test}, and the control well is marked as $A_{Control}$. Finally calculate $\Delta A_{Test}=A_{Test}-A_{Control}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.

Note: Blank well only needs to measure 1 time. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. The ΔA_{Test} of the sample ranges from 0.012 to 1.2. If the ΔA_{Test} of the sample is greater than 1.2, the sample needs to be appropriately diluted with the Extraction Buffer before measurement, and the calculated y value is multiplied by the dilution factor. Each sample needs to set up a control well, and it is measured immediately after the color development is completed, and the absorbance value will decrease after 2 h.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculation of OPC content

Bring the ΔA_{Test} of the sample into the equation to get the y value (mg/mL).

(1) Calculated by fresh weight of samples

OPC (mg/g weight)=$y\times V_{Extraction\ Buffer}\div W$**$=y\div W$**

(2) Calculated by protein concentration

OPC (mg/mg prot) =$y\times V_{Extraction\ Buffer}\div(Cpr\times V_{Extraction\ Buffer})$**$=y\div Cpr$**

Where: $V_{Extraction\ Buffer}$: Extraction Buffer added, 1 mL; Cpr: sample protein concentration, mg/mL; W: Sample weight, g.

Typical Data

Typical standard curve:

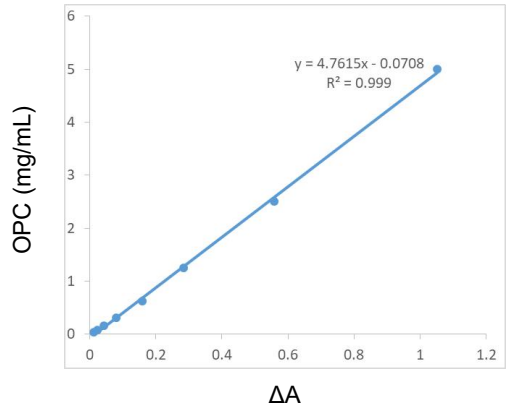

Figure 1. Standard curve for OPC.

Recommended Products

Catalog No.	Product Name
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1080	CheKine™ Micro Superoxide Anion Scavenging Capacity Assay Kit
KTB1091	CheKine™ Micro Hydroxyl Free Radical Scavenging Capacity Assay Kit
KTB1510	CheKine™ Micro Uric Acid (UA) Assay Kit
KTB1530	CheKine™ Micro Plant Flavonoids Assay Kit
KTB1540	CheKine™ Micro Plant Total Phenols (TP) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.