Proanthocyanidin

Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1235

(Version 1.2A)

Detection and Quantification ofProanthocyanidinContentin Tissue extractsand Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Proanthocyanidins are a class of polyphenols found in a variety of plants. Chemically, they are oligomeric flavonoids. Many are oligomers of catechin and epicatechin and their gallic acid esters. More complex polyphenols, having the same polymeric building block, form the group of tannins.

ProanthocyanidinColorimetric Microplate Assay Kit provides a convenient tool for sensitive detection of proanthocyanidin in a variety of samples. The proanthocyanidin is subsequently measured by a coupled chemical reaction system with a colorimetric readout at 500 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	8 ml x 1	4 °C
Dye Reagent	Powderx 1	4 °C
Dye Reagent Diluent	10 ml x 1	4 °C
Standard	Powder x 1	4 °C
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Note:

Standard: add 1 ml Assay Buffer to dissolve before use, the concentration will be 10 mmol/L.

Dye Reagent: add 10 ml Dye Reagent Diluent to dissolve before use.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 500 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer

IV. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer, then transfer it to the microcentrifuge tubes; incubate at 60 °C water bath for1 hour; centrifuged at 10,000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2.For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	20 µl	--	--
Standard	--	20 µl	--
Assay Buffer	--	--	20 µl
Reaction Buffer	80 µl	80 µl	80 µl
Dye Reagent	100 µl	100 µl	100 µl
Mix, incubate at 37 °C for 20minutes, record absorbance measured at 500 nm.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the weight of sample

Proanthocyanidin (mmol/g) $=(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay})$

$=0.01 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

2. According to the volume of sample

Proanthocyanidin (mmol/ml) $=(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$

$= 0.01 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

$C_{Standard}$: the concentration of standard, 10 mmol/L = 0.01 mmol/ml;

W: the weight of sample, g;

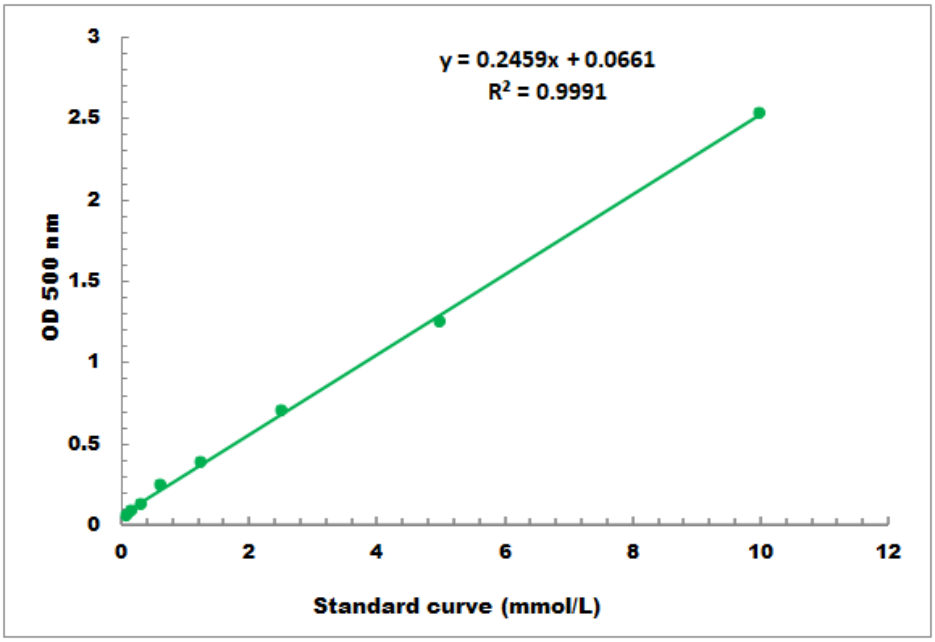
$V_{Standard}$: the volume of standard, 0.02 ml;

V_{Sample}: the volume of sample, 0.02 ml;

V_{Assay}: the volume of Assay Buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.1 mmol/L - 10 mmol/L

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES