Total PhenolsMicroplate Assay Kit

User Manual

Catalog # CAK1223

(Version 1.4A)

Detection and Quantification of Total PhenolsContent inUrine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture and Other biological fluidsSamples.

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

I. INTRODUCTION

Phenols constitute probably the largest group of plant secondary metabolites, varying in size from a simple structure with an aromatic ring to complex ones such as lignins. Although many of the essential oils are terpenes, some are phenolic compounds. Many simple phenols are responsible for taste. They are called the phenylpropanoids because they originate from phenylalanine and they have a six-carbon (C6) and three-carbon (C3) structure.

Total Phenols Microplate Assay Kitis a sensitive assay for determining total phenols content in various samples.Phenols can react with phosphomolybdic acid, and the product can be measured at a colorimetric readout at 760 nm, is proportional to the phenols concentration in the sample.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	3 ml x 1	4 °C
Dye Reagent	1 ml x 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
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Note:

Standard: add 1 ml Assay Bufferto dissolve before use, then add 0.2 ml into 0.8 ml Assay Buffer, mix; the concentration will be 4mmol/L.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 760 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay Buffer, put it in water bath of 60°C for 2 hours with shaking, centrifuged at 10,000g for 10minutes, take the supernatant into a new centrifuge tube.

2.For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Warm theReaction Buffer, Dye Reagentto room temperature before use.

Add following reagents intothe microplate:

Reagent	Sample	Standard	Blank
Sample	10µl	--	--
Standard	--	10 µl	--
Distilled water	120µl	120µl	130µl
Reaction Buffer	60µl	60 µl	60 µl
Mix, stay at room temperature for 5 minutes.			
Dye Reagent	10 µl	10 µl	10 µl
Mix,stay at room temperature for 10 minutes,measured at 760 nm and record the absorbance.			

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 volume of sample

Total Phenols (μmol/ml) $=(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V_{Sample}$

$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank})$

2. According to the weight of sample

Total Phenols (μmol/g) $=(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay})$

$= 4 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$

$C_{Standard}$: the standard concentration, 4 mmol/L = 4μmol/ml;

W: the weight of sample, g;

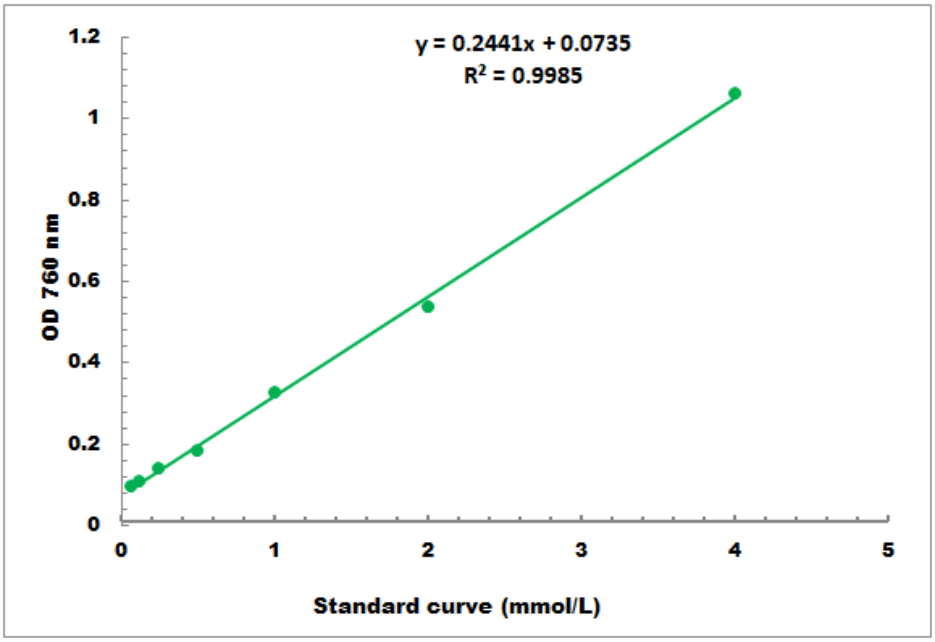
V_{Assay}:the volume of Assay Buffer, 1 ml;

V_{Sample}: the volume of sample, 0.01 ml;

$V_{Standard}$: the volume of standard, 0.01 ml.

VII. TYPICAL DATA

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



Detection Range: 0.04 mmol/L - 4 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