Total Phenols

Colorimetric Microplate 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 Colorimetric 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 keep in dark
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

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. REAGENT PREPARATION

Standard:Briefly centrifuge prior to opening.Dissolvein1 ml Assay Bufferto generate 20 mmol/L of standardstock solution, store at 4 °C for 1 monthafter reconstitution.Then dilute to 4 mmol/Lstandard top solutionby adding0.2 ml stock solution into 0.8 mlAssay Buffer.Perform 2-fold serial dilutions of the top standardsolution using Assay Bufferto make the standard curve.The concentration of standard curve could be4/2/1/0.5/0.25/0.125/0.0625 mmol/L.

V. 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.

VI. ASSAY PROCEDURE

Warm theReaction Buffer, Dye Reagentto room temperature before use.

Add following reagents intothe microplate:

Reagent*	Sample**	Standard	Blank
Distilled water	120µl	120µl	130µl
Reaction Buffer	60µl	60 µl	60 µl
Sample	10 µl	--	--
Standard	--	10 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:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

**For colored samples, we recommend setting a parallel sample background control well with same volume of sample only. Other reagents were replaced by distilled water to the same total volume. Subtract the OD value of the sample background control from the OD value of the sample to correct for interference from the sample's own color.

VII. CALCULATION

1. Calculatethe sample concentration in ASSAY PROCEDURE according to the slope of the standard curve

$$C=\frac{(OD_{Sample}-OD_{Blank})-\text{Intercept}}{\text{Slope}}\times n(mmol/L)$$

Calculate the initial concentration according to sample preparation procedure.

2. According to one point of the standard OD and concentration

2.1 According to the weight of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times W\times(V_{Sample}/V_{Assay})}(\mu mol/g)$$

2.2According to the volume of sample

$$C=\frac{(C_{Standard}\times V_{Standard})\times(OD_{Sample}-OD_{Blank})}{(OD_{Standard}-OD_{Blank})\times V_{Sample}}(\mu mol/ml)$$

Slope: the absorbance slope of standard curve

n: the dilution factor

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

$V_{Standard}$: the volume of standard in assay procedure, μl

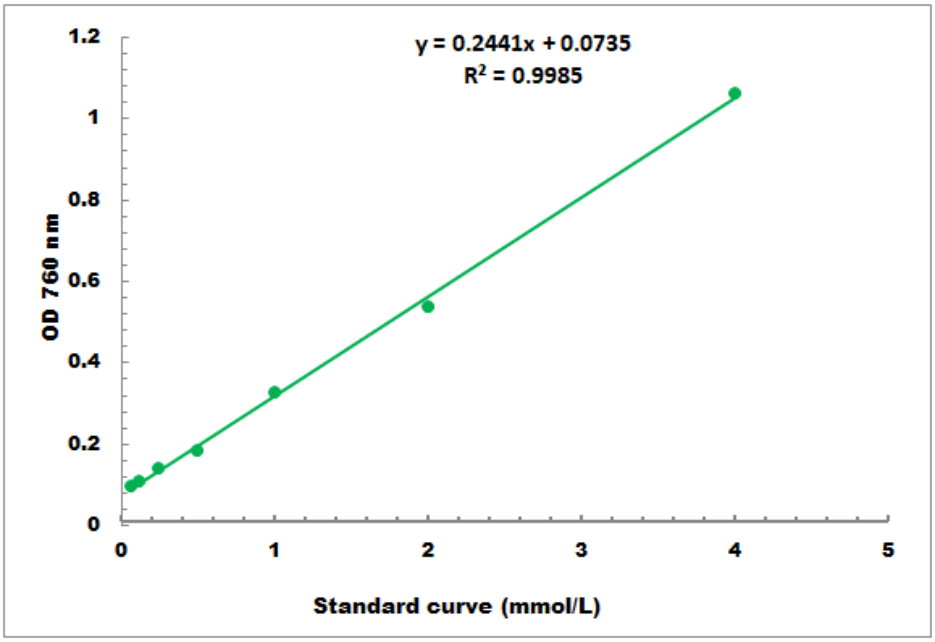
V_{Sample}: the volume of sample in assay procedure, μl

V_{Assay}: the volume of Assay Buffer, μl

W: the weight of sample, g

VIII. 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