Polyphenol Oxidase Activity

ColorimetricMicroplate Assay Kit

User Manual

Catalog # CAK1013

(Version 1.3F)

Detection and Quantification of Polyphenol Oxidase(PPO) Activity in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluids Samples.

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

I. INTRODUCTION

Polyphenol oxidase is a bifunctional, copper-containing oxidase having catecholase and cresolase activity. It is responsible for browning reactions through the phylogenetic scale.

Polyphenol OxidaseActivity Colorimetric Microplate Assay Kit is a sensitive assay for determining polyphenol oxidase activity in various samples. The assay is initiated with the enzymatic hydrolysis of the catechol by polyphenol oxidase. The enzyme catalysed reaction productsquinone, can be measured at a colorimetric readout at 410 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	30 ml x 1	4 °C
Substrate	Powder x 1	4 °C, keep in dark
Stop Solution	20 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 410 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Ice
7. Centrifuge
8. Timer

IV. REAGENT PREPARATION

Substrate: Add 10 ml distilled water to dissolve before use.Prepare for immediate use or -20°C for 2 weeksafter reconstitution.

Positive Control: Briefly centrifuge prior to opening. Add 0.5 ml distilled waterto dissolve before use. Store at -80°C for 1 month after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

V. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1g tissue, homogenize with 1mlAssay Buffer on ice, centrifuged at 8000g 4°C for 10minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For serum, plasma samples or plant juice

Add 0.1 ml serum, plasmaor plant juice into 0.9 ml Assay Buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

VI. ASSAY PROCEDURE

WarmReaction Buffer and Substrateto 37°C before use.

Add following reagents intothe microcentrifuge tubes:

Reagent*	Sample**	Control**	Positive Control
Sample	50 µl	--	--
Sample (boiled)	--	50 µl	--
Positive Control	--	--	50 µl
Reaction Buffer	150µl	150µl	150µl
Substrate	50µl	50µl	50µl
Mix, put it in the oven,37°Cfor 3minutes.			
Stop Solution	100 µl	100 µl	100 µl
Mix, centrifuged at 10000gfor 5 minutes, add 200µlsupernatant into the microplate, record absorbance measured at 410 nm.			

Note:

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

**For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

**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

Unit Definition: one unit is defined as the OD valuechanged 0.01per minute in the reaction system.

1.1 According to the protein concentration of sample

$$\text{Activity} = \frac{V_{Total} \times (OD_{Sample} - OD_{Control})}{V_{Sample} \times C_{Protein} \times T \times 0.01} (U/mg)$$

1.2 According to the weight of sample

$$\text{Activity} = \frac{V_{Total} \times (OD_{Sample} - OD_{Control})}{W \times (V_{Sample} / V_{Assay}) \times T \times 0.01} (U/g)$$

1.3 According to the volume of sample

$$\text{Activity} = \frac{V_{Total} \times (OD_{Sample} - OD_{Control})}{V_{Sample} \times V / (V + V_{Assay}) \times T \times 0.01} (U/mL)$$

1.4 According to the quantity of cell or bacteria

$$\text{Activity} = \frac{V_{Total} \times (OD_{Sample} - OD_{Control})}{N \times (V_{Sample} / V_{Assay}) \times T \times 0.01} (U/10^4)$$

$C_{Protein}$: the protein concentration, mg/ml

W: the weight of sample, g

V: the volume of sample in sample preparation, ml

N: the quantity of cell or bacteria, $N \times 10^4$

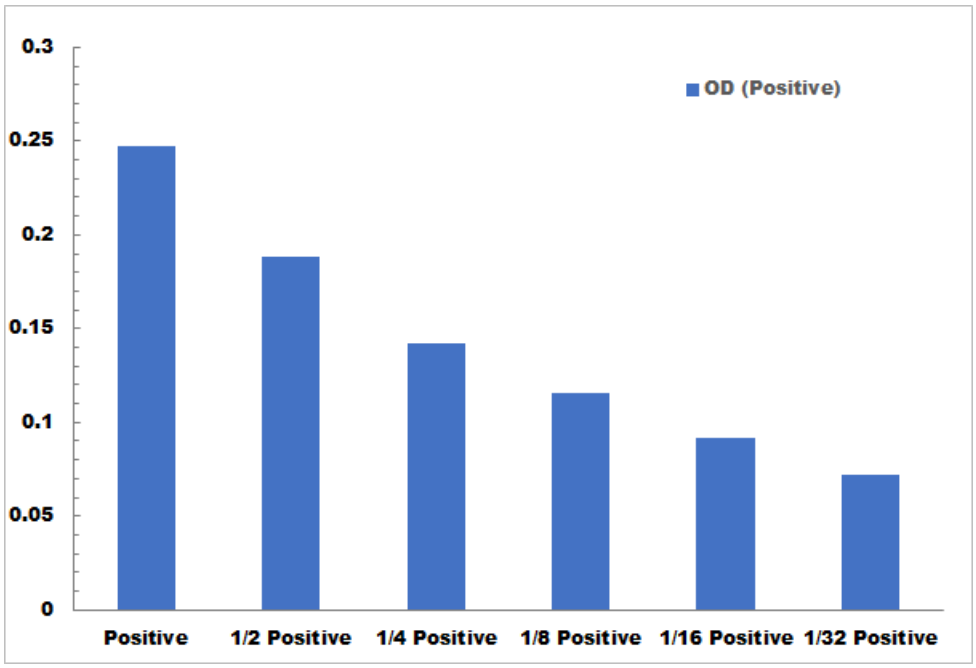
V_{Total}: thetotal volume of all reagents in assay procedure, ml

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

V_{Assay}: the volume of Assay Buffer in sample preparation, ml

T: the reaction time, minute

VIII. TYPICAL DATA



Positive Control reaction in 96-well plate assay with decreasing the concentration