Polyphenol Oxidase

Microplate Assay Kit

User Manual

Catalog # CAK1013

(Version 1.2F)

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 Oxidase 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 products quinone, 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
Stop Solution	20 ml x 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 Strips	
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Note:

Substrate: add 10 ml distilled water to dissolve before use.

Positive Control: add 0.5 ml distilled water to dissolve before use.

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. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay 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 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 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.

3. For serum, plasma samples or plant juice

Add 0.1 ml serum, plasma or 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.

V. ASSAY PROCEDURE

Warm Reaction Buffer and Substrate to 37 °C before use.

Add following reagents into the microcentrifuge tubes:

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

Note:

1) 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 sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

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

VI. CALCULATION

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

1. According to the protein concentration of sample

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

$$= 233.3 \times (OD_{Sample} - OD_{Control}) / C_{Protein}$$

2. According to the weight of sample

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

$$= 233.3 \times (OD_{Sample} - OD_{Control}) / W$$

3. According to the quantity of cell or bacteria

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

$$= 233.3 \times (OD_{Sample} - OD_{Control}) / N$$

4. According to the volume of serum, plasma or plant juice

$$PPO\ (U/ml) = (OD_{Sample} - OD_{Control}) \times V_{Total} / (V \times V_{Sample} / V_{Assay}) / 0.01 / T$$

$$= 233.3 \times (OD_{Sample} - OD_{Control}) / V$$

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

W: the weight of sample, g;

V: the volume of sample, ml;

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

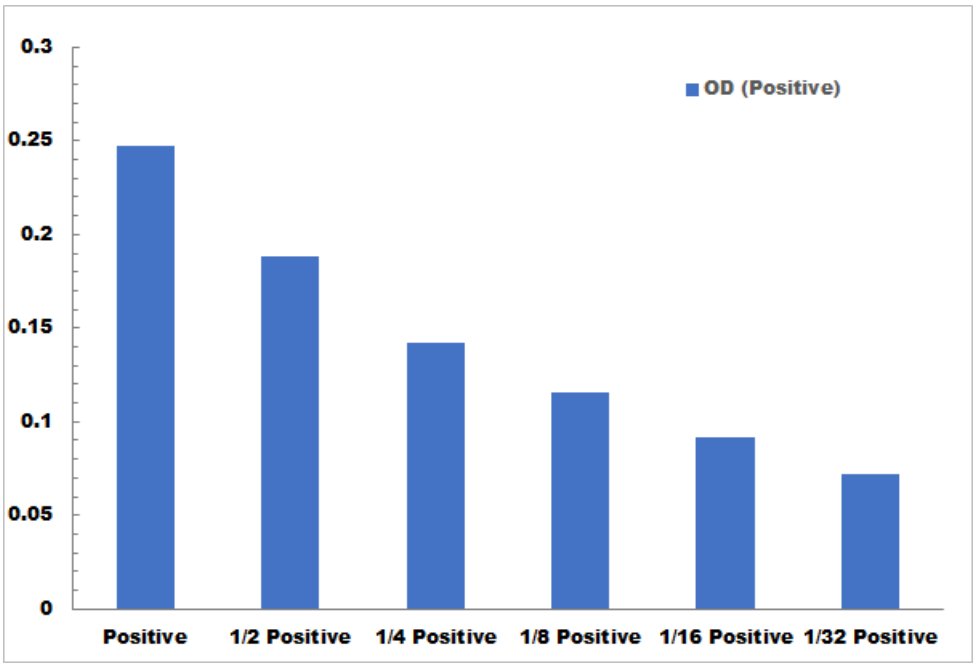
V_{Total}: the volume of sample, 0.35 ml;

V_{Sample}: the volume of sample, 0.05 ml;

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

T: the reaction time, 3 minutes.

VII. TYPICAL DATA



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

VIII. TECHNICAL SUPPORT

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

IX. NOTES