Polyphenol Oxidase Assay Kit

Polyphenol Oxidase Assay Kit is a detection kit for the quantification of Polyphenol Oxidase Activity in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82015

Package: 96 wells

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INTRODUCTION

Polyphenol oxidase (PPO; also polyphenol oxidase i, chloroplastic), an enzyme involved in fruit browning, is a tetramer that contains four atoms of copper per molecule, and binding sites for two aromatic compounds and oxygen.

PPO may accept monophenols and/or o-diphenols as substrates. The enzyme works by catalyzing the o-hydroxylation of monophenol molecules in which the benzene ring contains a single hydroxyl substituent to o-diphenols (phenol molecules containing two hydroxyl substituents at the 1, 2 positions, with no carbon between). It can also further catalyse the oxidation of o-diphenols to produce o-quinones. PPO catalyses the rapid polymerization of o-quinones to produce black, brown or red pigments (polyphenols) that cause fruit browning. The amino acid tyrosine contains a single phenolic ring that may be oxidised by the action of PPOs to form o-quinone. Hence, PPOs may also be referred to as tyrosinases.

Common foods producing the enzyme include mushrooms (*Agaricus bisporus*), apples (*Malus domestica*), avocados (*Persea americana*), and lettuce (*Lactuca sativa*). [Wikipedia: Polyphenol Oxidase]

PRINCIPLE OF THE ASSAY

The Polyphenol Oxidase Assay Kit is used for determining Polyphenol Oxidase activity in various samples. Catechol hydrolysis by Polyphenol Oxidase and the enzyme catalyzed reaction products quinone, and it can be measured at 410nm. The Polyphenol Oxidase activity in the sample is then determined by comparing the O.D. of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Upon received, store the positive control at -20 °C and store other component in the kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate	1 X 96-well plate	4°C
Assay Buffer	4 X 30 ml (ready to use)	4°C
Reaction Buffer	30 ml (ready to use)	4°C
Substrate	1 vial (Lyophilized)	4°C
Positive control	1 vial (Lyophilized)	-20°C
Stop Solution	20 ml (ready to use)	4°C
Plate sealer	3 strips	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 410 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Convection oven (37°C)
- Water bath or heating block

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store the positive control at -20 °C and store other component in the kit at 2-8°C.
- Briefly spin down the reagents before use.

- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Liquid Sample Preparation (for serum, plasma samples or plant juice samples)

Add **0.1 ml** of **liquid samples** into **0.9 ml** of **Assay buffer** in a microcentrifuge tube on ice, mix well. Centrifuge the tube 8000X g at 4 °C for 10 minutes, collect the supernatant into a new tube and keep it on ice before assay.

Cell lysate- Collect cell in a centrifuge tube, wash 1-2X by PBS. Discard the supernatant after centrifugation, add 1 ml of Assay buffer pre 5×10^6 cells in the tube. And then sonicate samples (set with power 20%, sonicate for 3 sec. and interval for 10 sec., repeat 30 times). Centrifuge samples 8000X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay.

Tissue lysate- Weigh out 0.1 g of tissue, homogenize with 1 ml Assay buffer on ice. Centrifuge samples 8000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay.

Note: Assay the samples immediately or aliquot and store the undiluted samples at -80°C for up to 2 weeks. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Substrate:** Reconstitute the Substrate with **10 ml** of **distilled water**. Allow the Substrate to sit for few minutes with gentle agitation to make sure the Substrate is dissolved completely before use. The reconstituted Substrate can be stored at 4°C for up to 1 week.
- **Positive control:** Reconstitute the Positive control with **0.5 ml** of **distilled water**. Allow the Positive control to sit for few minutes with gentle agitation to make sure the Positive control is dissolved completely before use. Aliquot and store the reconstituted Positive control at -80°C.

ASSAY PROCEDURE

Warm Reaction Buffer and Substrate to 37 °C before use. Controls and samples should be assayed in at least duplicates.

1. Add **50 µl** per **(diluted) samples** into each new microcentrifuge tube.
2. Add **50 µl** of **boiled Sample from step 1** into each control tube.
3. Add **50 µl** of **Positive control** into each Positive control tube.
4. Add **150 µl** of **Reaction Buffer** per tube into each Sample and Control tube.
5. Add **50 µl** of reconstituted **Substrate** into each Sample and Control tube.
6. Mix well and incubate all tubes at **37°C for 3 min.**
7. Then put all tubes on ice immediately.
8. Add **100 µl** of **Stop Solution** per tube into each Sample and Control tube.
9. Mix well and **centrifuge all tubes at 10000X g for 5 minutes**.
10. Transfer **200 µl** of the supernatant from each tube into the microplate.
11. Read the OD with a microplate reader at **410 nm** immediately.

Summary of Assay Procedure (reaction is in tube before transfer to plate)

Reagent	Sample	Control	Positive Control
Sample	50 µl	-	-
Boiled Sample	-	50 µl	-
Positive Control	-	-	50 µl
Reaction Buffer	150 µl	150 µl	150 µl
Substrate	50 µl	50 µl	50 µl
Mix well and incubate all tubes at 37°C for 3 min			
Put all tubes on ice immediately.			
Stop Solution	100 µl	100 µl	100 µl
Mix well and centrifuge all tubes at 10000X g for 5 minutes.			
Transfer 200 µl of the supernatant from each tube into the microplate			
Read the OD with a microplate reader at 410 nm immediately			

CALCULATION OF RESULTS

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

2. Calculate the average absorbance values for each set of samples, standards, controls and blank.

2. Calculation:

A. Definition:

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

W: the weight of sample, g;

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

V_{Sample}: the volume of reaction sample, 50 µl = 0.05 ml;

V_{total}: the total volume of reaction, 350 µl = 0.35 ml;

V_{Assay}: the volume of total sample in Assay buffer, 1 ml;

V: the volume of liquid sample to be diluted in Assay buffer, 0.1 ml;

T: the reaction time, 3 minutes.

B. Formula:

a). According to the protein concentration of sample

Polyphenol Oxidase activity (U/mg) =

$[(OD_{Sample} - OD_{Control}) \times V_{total}] / [V_{Sample} \times C_{Protein} \times 0.01 \times T]$

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

b). According to the weight of sample

Polyphenol Oxidase activity (U/g) =

$[(OD_{Sample} - OD_{Control}) \times V_{total}] / [W \times (V_{Sample} / V_{Assay}) \times 0.01 \times T]$

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

c). According to the quantity of cells

Polyphenol Oxidase activity ($U/10^4$ cell) =

$[(OD_{Sample} - OD_{Control}) \times V_{total}] / [N \times (V_{Sample} / V_{total}) \times 0.01 \times T]$

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

d). According to the volume of sample

Polyphenol Oxidase activity (U/ml) =

$[(OD_{Sample} - OD_{Control}) \times V_{total}] / [V_{Sample} \times (V / V_{Assay}) \times 0.01 \times T]$

$= 2333.3 \times (OD_{Sample} - OD_{Control})$