# Pyruvate Oxidase Activity

# Colorimetric Microplate Assay Kit

# User Manual

## Catalog # CAK1316

(Version 1.1A)

Detection and Quantification of Pyruvate Oxidase(POX)Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media and Other biological fluidsSamples.

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

## I. INTRODUCTION

Pyruvate Oxidase(POX) is a thiamine pyrophosphate-dependent oxidase that catalyzes the oxidative decarboxylation of pyruvate to acetyl phosphate, carbon dioxide and water. Pyruvate oxidase is an important enzyme in bacterial metabolism and is often used in biochemical research.

Pyruvate Oxidase ActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring pyruvate oxidaseactivity in a variety of samples. Pyruvate is oxidized by pyruvate oxidase. The enzyme catalysed reaction products quinone can be measured at a colorimetric readout at 550 nm.

## II.KIT COMPONENTS

| Component | Volume | Storage |
|---|---|---|
| 96-Well Microplate | 1 plate | |
| Assay Buffer | 30mlx 4 | 4 °C |
| Reaction Buffer | 10 ml x 1 | 4 °C |
| Substrate | Powder x 1 | 4 °C |
| Dye Reagent | Powder x 1 | 4 °C, keep in dark |
| Standard (5 mmol/L) | 1 ml x 1 | 4 °C |
| Positive Control | Powder x 1 | -20 °C |
| Plate Adhesive Strips | 3 Strips | |
| Technical Manual | 1 Manual | |

**Note:**

**Substrate**: add 1 ml Reaction Bufferto dissolve before use, mix.Store at 4 °C. Use within one month.

**Dye Reagent**: add 10 ml Distilled waterto dissolve before use, mix.Store at -20 °C. Use within one month.

**Positive Control**: add 1 ml Assay Buffer to dissolve before use, mix.Aliquot & store at -20 °C.Use within one month.

## III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 550 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 cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly.

## V. ASSAY PROCEDURE

Warm all reagentsto room temperature before use.

Add following reagents intothe microplate:

| Reagent | Sample | Control | Standard | Blank | Positive Control |
|---|---|---|---|---|---|
| Reaction Buffer | 80 µl | 80 µl | 90 µl | 90 µl | 80 µl |
| Substrate | 10 µl | 10 µl | -- | -- | 10 µl |
| Sample | 10 µl | -- | -- | -- | -- |
| Standard | -- | -- | 10 µl | -- | -- |
| Distilled water | -- | 10 µl | -- | 10 µl | -- |
| Positive Control | -- | -- | -- | -- | 10 µl |
| Dye Reagent | 100 µl | 100 µl | 100 µl | 100 µl | 100 µl |
| Mix, put it in the oven,37 °C for 10 minutes, measured at 550 nm and recordthe absorbance. | | | | | |

**Note:**

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) 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.

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

## VI. CALCULATION

**Unit Definition**: One unit of pyruvate oxidase activity is defined asthe enzyme generates 1 μmol of H2O2 per minute.

1. According to the protein concentration of sample

$$POX\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein}) / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$POX\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cell or bacteria

$$POX\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$POX\ (U/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 0.5 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

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

$C_{Standard}$: the standard concentration, 5 mmol/L = 5μmol/ml;

W: the weight of sample, g;

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

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

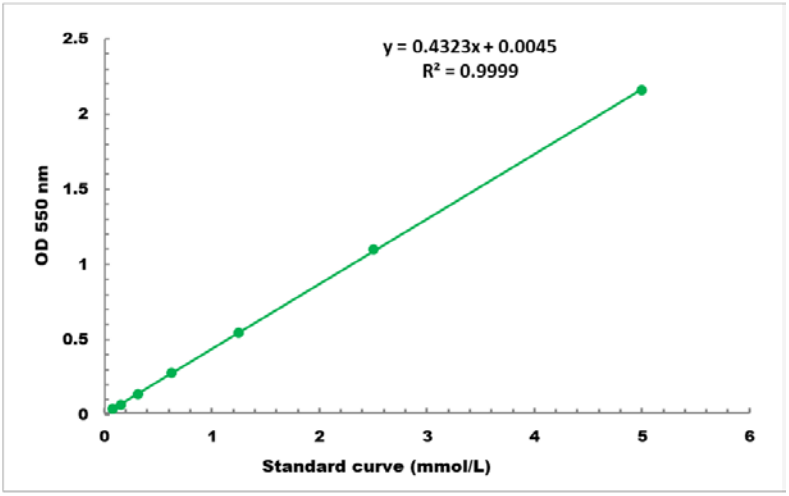
$V_{Standard}$: the volume of standard, 0.01 ml;

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

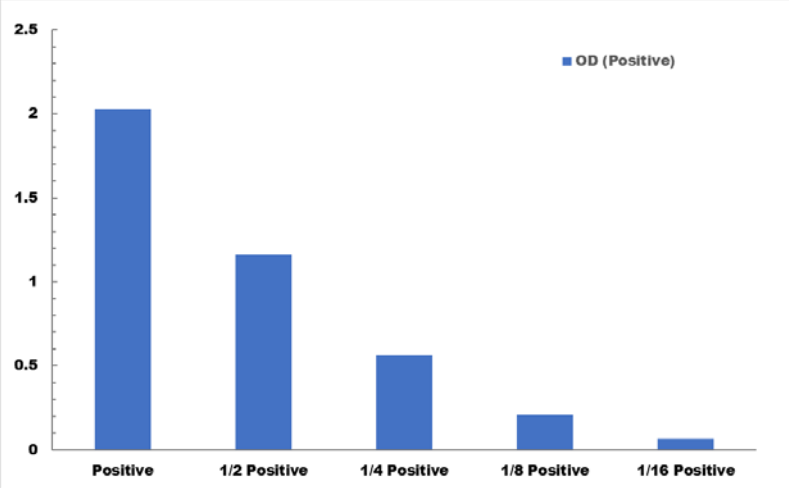
T: the reaction time, 10 minutes.

## VII. TYPICAL DATA

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




Detection Range: 0.05 mmol/L - 5 mmol/L




Positive Control reaction in 96-well plate assay with decreasing theconcentration

## VIII. TECHNICAL SUPPORT

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

## IX. NOTES