



Pyruvate Oxidase Activity Colorimetric Microplate Assay Kit User Manual

Catalog # CAK1316

(Version 1.2A)

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.

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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 (5mmol/L)	1 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 550 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. Perform 2-fold serial dilutions of the top standard solution using distilled water to make the standard curve. The concentration of standard curve could be 5/2.5/1.25/0.625/0.312/0.156/0.078 mmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 ml Reaction Buffer to dissolve before use, mix. Store at 4 °C for 2-3 weeks or -20 °C for 5-6 months after reconstitution.

Dye Reagent: Add 10 ml Distilled water to dissolve before use, mix. Keep in dark and store at 4 °C for 2-3 weeks or -20 °C for 2 months.

Positive Control: Briefly centrifuge prior to opening. Add 1 ml Assay Buffer to dissolve before use, mix. Aliquot & store at -20 °C and use within 1 month.

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 ml Assay Buffer for 5×10^6 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

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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.

VI. ASSAY PROCEDURE

Warm all reagents to room temperature before use.

Add following reagents into the microplate:

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

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 of pyruvate oxidase activity is defined as the enzyme generates 1 μmol of H_2O_2 per minute.

1. According to the slope of the standard curve

$$\text{Activity} = \frac{(\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) - \text{Intercept}}{\text{Slope} \times T} \times n (\text{U/mL})$$

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

2.1 According to the protein concentration of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times C_{\text{Protein}} \times T} (\text{U/mg})$$

2.2 According to the quantity of cells or bacteria

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times N \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U}/10^4)$$

2.3 According to the weight of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times W \times (V_{\text{Sample}} / V_{\text{Assay}}) \times T} (\text{U/g})$$

2.4 According to the volume of sample

$$\text{Activity} = \frac{(C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}})}{(\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \times V_{\text{Sample}} \times T} (\text{U/mL})$$

Slope: the absorbance slope of standard curve

n: the dilution factor

C_{Protein} : the protein concentration of sample, mg/mL

W: the weight of total sample, g

N: the quantity of total cell or bacteria sample, 10^4

C_{Standard} : the concentration of standard, $\mu\text{mol/mL}$

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

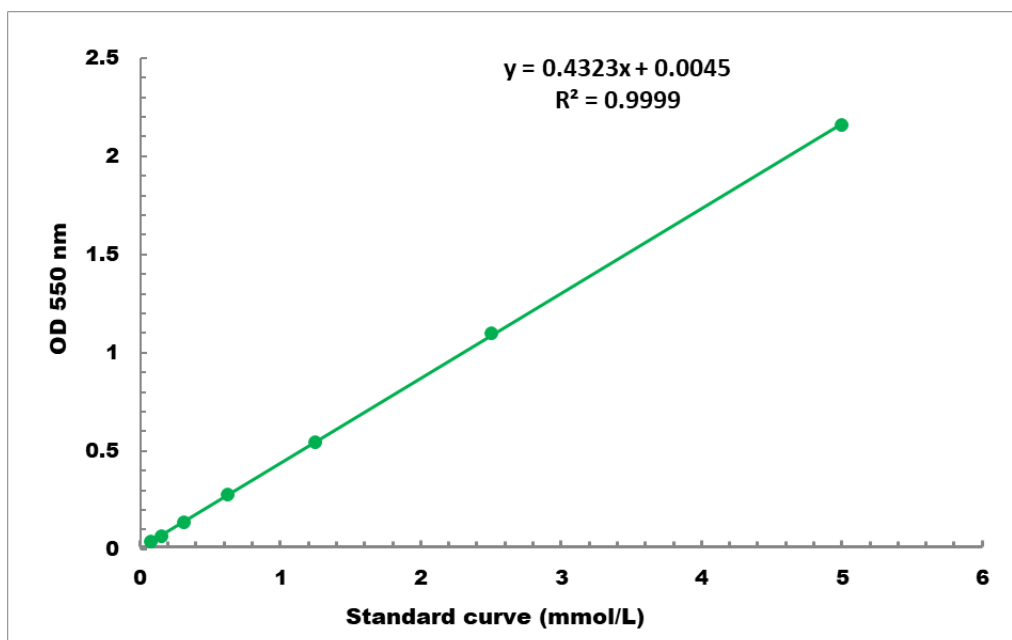
V_{Sample} : the volume of sample in assay procedure, mL

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

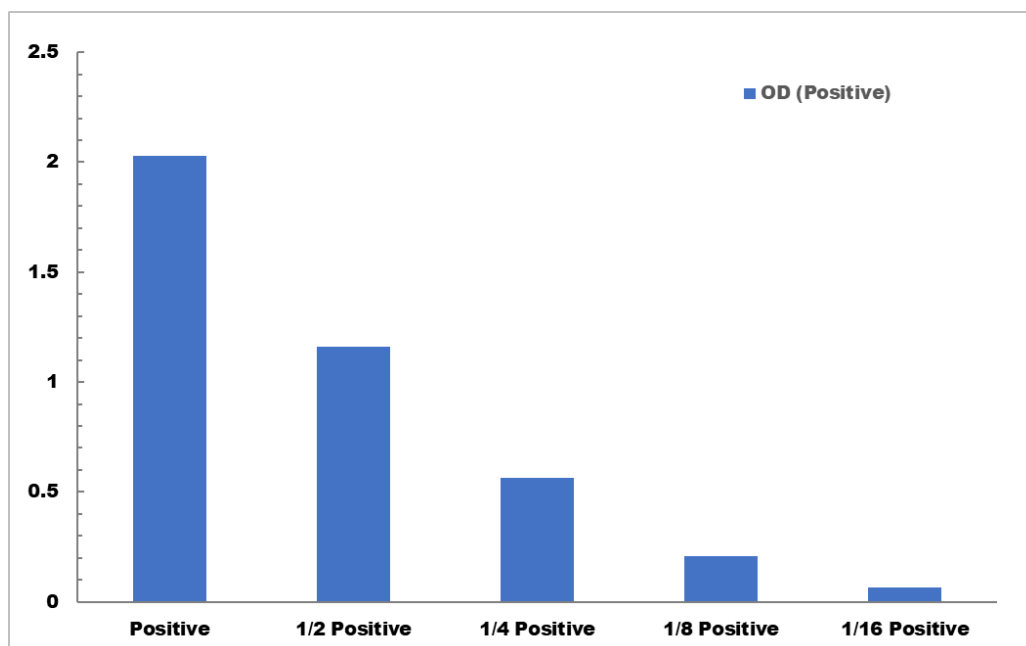
T: the reaction time, minute

VIII. TYPICAL DATA

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 the concentration