Pyruvate Oxidase Activity Fluorometric Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6895

Detection and Quantification of Pyruvate Oxidase Activity in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluids Samples.

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

1. 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 Oxid ase Activity Microplate Assay Kit provides a simple and sensitive method for monitoring pyruvate oxidase activity in various samples. In this assay, pyruvate is oxidized by pyruvate oxidase , resulting in the generation of an intermediate that reacts with the probe , which can be detected fluorometrically (Ex/Em 535/587).

2. Kit Components

Component	Volume	Storage
96-Well Black Microplate	1 plate	-
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	20 mlx 1	4 °C
Substrate	Powderx 1	-20 °C
Enzyme	Powderx 1	-20 °C
Probe	Powder x 1	-20 °C, keep in dark
Probe Diluent	1 mlx 1	4 °C
Standard (100 µmol/L)	1 mlx 1	4 °C
Positive Control	Powder x 1	-20 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. Materials Required But Not Provided

1. Fluorescence microplate reader to read fluorescence at Ex/Em = 535/587
2. Distilled water
3. Pipettor , multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Perform 2 -fold serial dilutions of the top standardsolution using distilled water to make the standard curve. The concentration of standard curve could be 100/50/25/12.5/6.25/3.12/1.56μmol/L.

Substrate: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use, mix. Store at 4°C. Use within 1 month.

Enzyme: Briefly centrifuge prior to opening. Add 1 mL Reaction Buffer to dissolve before use.Aliquot & store at -20°C.Use within 1 month.

Probe: Warm Probe Diluent to RT prior to use to melt frozen Probe Diluent; then add 1 mL Probe Diluent to dissolve. Store at -20°C, protect from light and moisture. Use within 1 month.

Positive Control: Briefly centrifuge prior to opening. Add 1 mL Assay Buffer to dissolve before use, then add 0.1 mL into 0.9 mL Assay Buffer mix. Store at -80°C. Use within 1 month.

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

5. 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 10000 g 4°C for 20minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples Weigh 0.1 g tissue, homogenize with 1 mL Assay Buffer on ice, centrifuged at 10000 g 4°C for 2 0minutes , take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For liquid samples Detect directly or dilute with Assay Buffer.

6. Assay Procedure

Warm thesolution to room temperature before use . Add following reagents in to the microplate :

Reagent*	Sample**	Control	Standard	Blank	Positive Control
Reaction Buffer	160 μl	160 μl	170 μl	170 μl	160 μl
Sample	10 μl	--	--	--	--
Distilled water	--	10 μl	--	10 μl	--
Standard	--	--	10 μl	--	--
Positive Control	--	--	--	--	10 μl
Substrate	10 μl	10 μl	--	--	10 μl
Probe	10 μl	10 μl	10 μl	10 μl	10 μl
Enzyme	10 μl	10 μl	10 μl	10 μl	10 μl
Mix, put it in the oven, 37 °C for 10 minutes,protected from light, record fluorescence measured at Ex/Em = 535/587 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.

7. Calculation

Unit Definition:

One unit of Pyruvate Oxidase activity is defined as the enzyme produce 1 μmol H_2O_2 per minat 37° C .

1. According to the slope of the standard curve

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

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

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

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

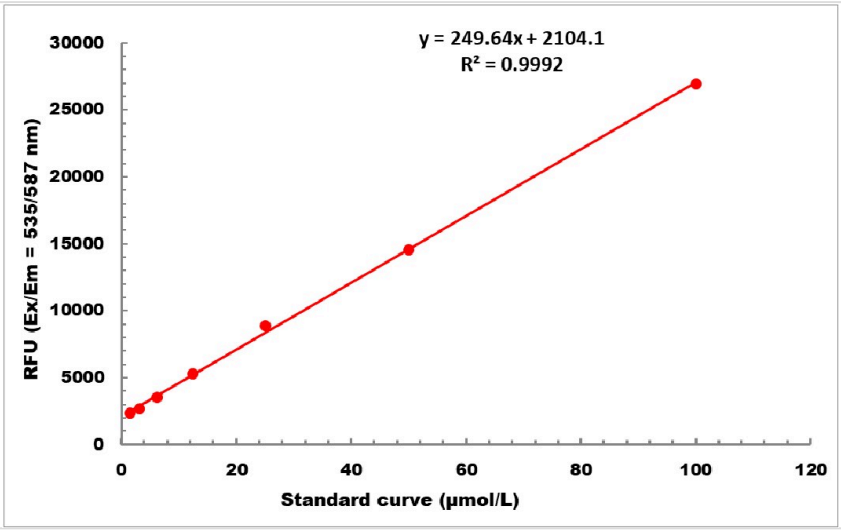
2.4 According to the volume of sample

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

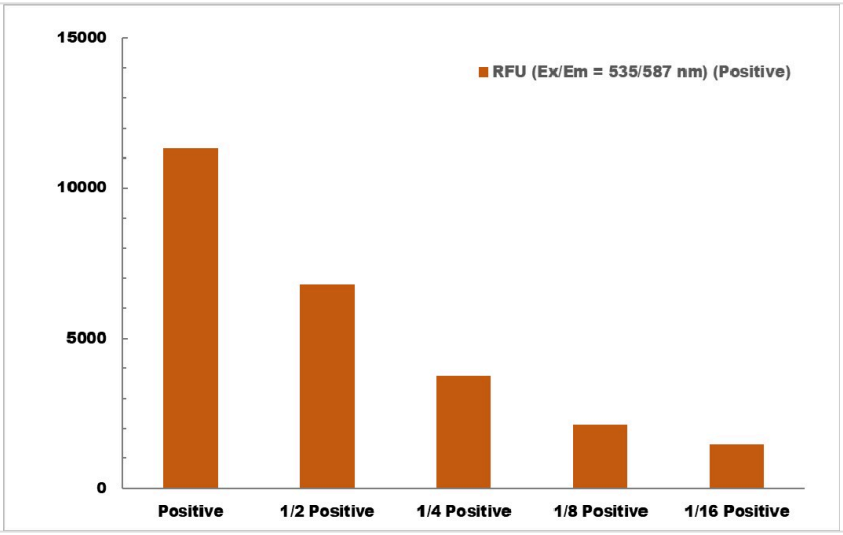
Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Protein}$	the protein concentration of sample, mg/mL
W	the weight of total sample , g
N	the quantity of total cell or bacteria sample, 10^4
$C_{Standard}$	the concentration of standard, μmol/mL
$V_{Standard}$	the volume 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

8. Typical Data

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



Detection Range : 1 μmol/L - 100 μmol/L



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