Pyruvate Dehydrogenase Activity Colorimetric Microplate Assay Kit

User Manual

Catalog # CAK1071

(Version 1.5G)

Detection and Quantification of Pyruvate Dehydrogenase(PDH) Activityin Tissue,Cultured Cell and Mitochondria isolates.

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

I. INTRODUCTION

Pyruvate dehydrogenase (PDH) is a mitochondrial enzyme that catalyzes the conversion of pyruvate to acetyl-CoA and CO2, and also links the tricarboxylic acid (TCA) and glycolysis pathways. The enzyme is inhibited by phosphorylation and activated by dephosphorylation. Mutations in PDH have been linked to pyruvate dehydrogenase deficiency (causing lactic acidosis and neurologic dysfunctions) and Leigh syndrome. PDH has also been implicated in oncogene induced senescence. PDH measurements can provide insights into metabolic functions and oncogenesis.

Pyruvate DehydrogenaseActivityColorimetric Microplate Assay Kit provides a simple and direct procedure for measuring pyruvate dehydrogenase activity levels in a variety of samples.The assay is initiated with the enzymatic hydrolysis of pyruvic acid by PDH. The enzyme catalyzed reaction products can be measured at a colorimetric readout at 450 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	25 mlx 4	4 °C, keep in dark
Reaction Buffer	5 mlx 1	4 °C
Substrate	Powderx 1	4 °C, keep in dark
Coenzyme Diluent	2 mlx 1	4 °C
Coenzyme	Powderx 1	-20 °C, keep in dark
Dye ReagentA	Powder x 1	4 °C, keep in dark
Dye Reagent B	1 ml x 1	4 °C, keep in dark
Positive ControlDiluent	0.5 mlx 1	4 °C
Positive Control	Powder x 1	-20 °C
Standard	Powder x 1	-20 °C, keep in dark
Technical Manual	1 Manual	

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 450 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. REAGENT PREPARATION

Substrate: Add 5 ml distilled waterand heat at 50°Cto dissolve completely before use. Keep in dark and store at 4°C for 1 week or -20 °C for 3 months.

Coenzyme: Briefly centrifuge prior to opening. Add 2ml Coenzyme Diluentto dissolve completely.Keep in dark and use immediately or store at -20 °C for 3 months.

Dye Reagent A: Add 5 mldistilled water to dissolve before use, mix, store at 4°Cfor 1 month.

Positive Control: Briefly centrifuge prior to opening. Dissolvein40 µl Positive Control Diluentbefore use.Store at -80 °C for 1 month.

Standard:Briefly centrifuge prior to opening. Dissolvein 1 mldistilled water to generate 2mmol/Lstock standard solution. Store stock solution at -20 °C for 2 weeks.Add 0.1 ml stock solutioninto 0.4 mldistilled water, the concentration will be 400 µmol/L as top standardsolution. Perform 2-fold serial dilutions of the top standardsolution using distilled waterto make the standard curve. The concentration of standard curve could be 400/200/100/50/25/12.5 µmol/L.

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

V. SAMPLE PREPARATION

1.For cell samples

Collect cell into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay Buffer for 5 ×10^6 cell (the quantity should be adjusted according to the actual situation of the sample), 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 Bufferon ice, centrifuged at 10000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For isolatedmitochondria

Mitochondria are isolated using a differential centrifugation method following gentle homogenization in an isotonic, detergent-free buffer containing sucrose or mannitol. Resuspend and Incubate mitochondriapellet with 1 mlAssay Buffer on ice for 15 minutesfor detection.

VI. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent*	Sample**	Control	Positive Control	Standard	Blank
Reaction Buffer	50 µl	50 µl	50 µl	50 µl	50 µl
Distilled Water	--	20 µl	--	40 µl	90 µl
Sample	20 µl	--	--	--	--
Positive Control	--	--	20 µl	--	--
Substrate	50 µl	50 µl	50 µl	--	--
Coenzyme	20 µl	20 µl	20 µl	--	--
Standard	--	--	--	50 µl	--
Dye Reagent A	50 µl	50 µl	50 µl	50 µl	50 µl
Dye ReagentB	10 µl	10 µl	10 µl	10 µl	10 µl
Mix, incubate at 37 °C for 15 minutes, measured at 450 nmand recordthe 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 PDHactivity is defined asthe enzyme produces 1μmolNADH per minute.

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

2.1According to the quantity of cells

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

2.2 According to the weight of sample

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

Slope: the absorbance slope of standard curve

n: the dilution factor

W: the weight of total sample, g

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

$C_{Standard}$: the concentration of standard, μmol/mL

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

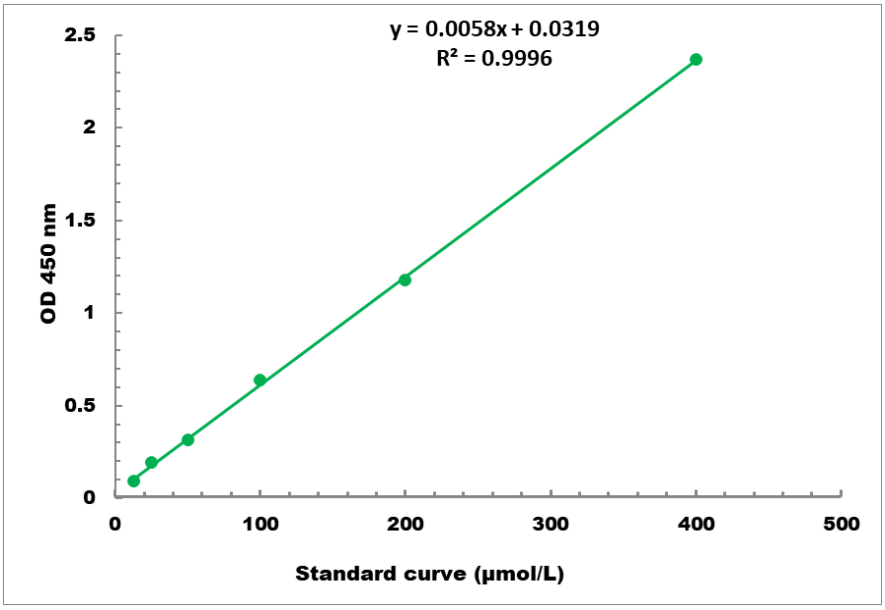
V_{Sample}: the volume of sample in assay procedure, mL

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

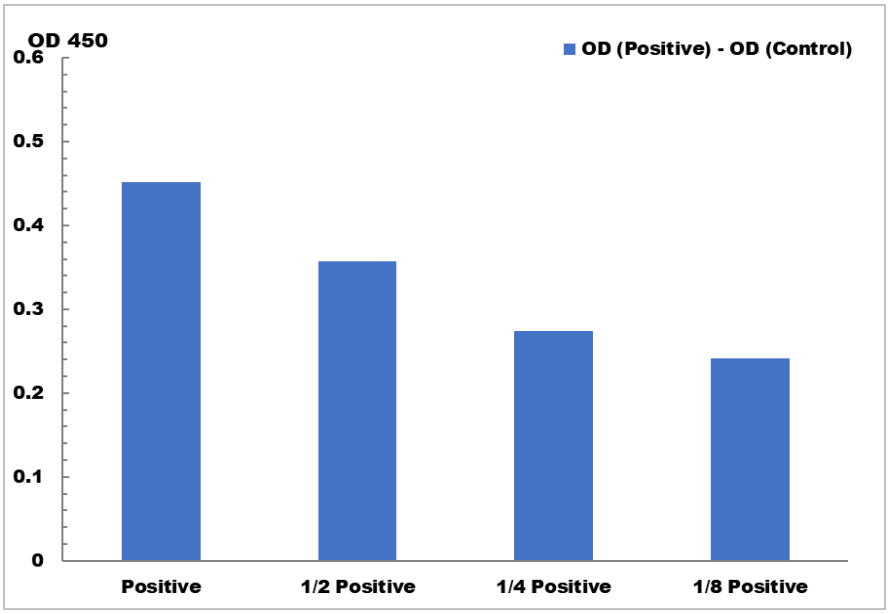
T: the reaction time, minute

VIII. TYPICAL DATA

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



Detection Range: 12.5μmol/L -400 μmol/L



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