Pyruvate Dehydrogenase Microplate Assay Kit

Basic information:

Catalog No.: UAK1071 **Size:** 100 Assays

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 CO_2, 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 oncogeneinduced senescence. PDH measurements can provide insights into metabolic functions and oncogenesis.

The assay is initiated with the enzymatic hydrolysis of Pyruvic acid by PDH. The enzyme catalysed reaction products can be measured at a colorimetric readout at 600 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer I	30 ml x 4	4 °C
Assay Buffer II	1.2 ml x 1	4 °C
Assay Buffer III	20 ml x 1	4 °C
Substrate Dilution	20 ml x 1	4 °C
Substrate	Powder x 1	4 °C
Technical Manual	1 Manual	

Note:

***Substrate**: add 19 ml Substrate Dilution to dissolve before use.*

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 600 nm

2. Distilled water

3. Pipettor

4. Pipette tips

5. Mortar

6. Centrifuge

7. Timer

8. Ice

IV. SAMPLE PREPARATION

1. For serum or plasma samples

Add 0.9 ml Assay Buffer I for 0.1 ml serum or plasma; mix; centrifuged at 8000g 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 I on ice, centrifuged at 11000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For mitochondria

Weigh out 0.1 g tissue, homogenize with 0.99 ml Assay Buffer I and 10 µl Assay Buffer II on ice, centrifuged at 600g 4 °C for 5 minutes. Take the supernatant into a new centrifuge tube, 11000g 4 °C for 10 minutes, discard the supernatant. Add 198 µl Assay Buffer III and 2 µl Assay Buffer II to the precipitation, shock, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times). Centrifuged at 11000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Warm Substrate to room temperature before use.

Add following reagents into the microplate:

Reagent	Sample
Sample	10 µl
Substrate	190 µl
Mix, measured at 600 nm and record the absorbance of 20th second and 80th second.	

VI. CALCULATION

Unit Definition: One unit of PDH activity is defined as the enzyme reduces 1 nmol of DCPIP per minute.

1. According to the protein concentration of sample

$$PDH\ (U/mg) = (OD_{Sample(20S)} - OD_{Sample(80S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (V_{Sample} \times C_{Protein}) / T$$

$$= 1587 \times (OD_{Sample(20S)} - OD_{Sample(80S)}) / C_{Protein}$$

2. According to the weight of sample

$$PDH\ (U/g) = (OD_{Sample(20S)} - OD_{Sample(80S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (W \times V_{Sample} / V_{Assay}) / T$$

$$= 317 \times (OD_{Sample(20S)} - OD_{Sample(80S)}) / W$$

3. According to the quantity of cells or bacteria

$$PDH\ (U/10^4) = (OD_{Sample(20S)} - OD_{Sample(80S)}) / (\varepsilon \times d) \times V_{Total} \times 10^9 / (N \times V_{Sample} / V_{Assay}) / T$$

$$= 317 \times (OD_{Sample(20S)} - OD_{Sample(80S)}) / N$$

ε: molar extinction coefficient, 21×10^3 L/mol/cm;

d: the optical path of 96-Well microplate, 0.6 cm;

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

W: the weight of sample, g;

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

V_{Total}: the total volume of the enzymatic reaction, 0.2 ml;

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

V_{Assay}: the volume of Assay buffer in sample preparation, 0.2 ml;

T: the reaction time, 1 minute.