Pyruvate Dehydrogenase(PDH) Activity Assay Kit

Product Code: 112025

Pyruvate Dehydrogenase (PDH) Activity Assay Kit - Microplate Method

Product Introduction

Pyruvate dehydrogenase (PDH, EC 4.1.1.1) is widely present in animals, plants, microorganisms, and cultured cells. It is part of the pyruvate dehydrogenase complex (PDHC), a rate-limiting enzyme complex that catalyzes the oxidative decarboxylation of pyruvate. This reaction generates hydroxyethyl-TPP and links glycolysis with the tricarboxylic acid cycle.

In this assay, PDH catalyzes the dehydrogenation of pyruvate while reducing WST-8 to produce a yellow product, resulting in increased absorbance at 450 nm.

Assay sample used for reference data: corn. Actual readings may vary depending on the testing instrument and assay conditions.

Product Packing List

Kit Size	Code	Component	Quantity
100T	112025.1	Reagent 1	100 mL
100T	112025.2	Reagent 2	1.5 mL
100T	112025.3	Reagent 3	20 mL
100T	112025.4	Reagent 4	17.4 mg
100T	112025.5	Reagent 5	2.5 mL
100T	112025.m	Instruction Manual	1 copy

Quality Standards and Safety Information

Raw Material or Packaging Name	Quality Standard	Main Toxicity
Reagent 1	--	--
Reagent 2	--	--
Reagent 3	--	--
Reagent 4	--	--
Reagent 5	--	--

Transportation and Storage

Transport	Transport with ice packs.
Storage	Store Reagents III and V at 2-8°C protected from light. Store the other reagents at -20°C.
Shelf Life	180 days.

Instructions for Use

1. Sample Pretreatment

1. **Tissue samples:** Weigh approximately 0.1 g tissue. Add 1 mL Reagent I and 10 uL Reagent II. Grind and homogenize thoroughly using an ice-bath homogenizer or mortar. Centrifuge at 11000 g, 4°C for 10 min. Collect the supernatant and keep it on ice for testing.
2. **Cell or bacterial samples:** Collect 500 ten thousand bacteria or cells into a centrifuge tube. Centrifuge and discard the supernatant. Add 1 mL Reagent I and 10 uL Reagent II. Disrupt the bacteria or cells by ultrasonic treatment in an ice bath using

200 W power, 3 s ultrasound, 7 s interval, for a total time of 5 min. Centrifuge at 11000 g, 4°C for 10 min. Collect the supernatant and keep it on ice for testing.

3. **Serum, plasma, and other liquid samples:** Test directly. If the solution is turbid, centrifuge and collect the supernatant for measurement.

2. Reagent Preparation

Before use, add 10 mL distilled water to Reagent IV to dissolve it. Store unused portions at -20°C.

3. Measurement Procedure

1. Preheat the microplate reader for 30 min or more and set the wavelength to 450 nm.
2. Prepare the working solution immediately before use according to the number of samples. Mix Reagent III, Reagent IV, and Reagent V at a ratio of 100 uL : 60 uL : 20 uL.
3. Add 20 uL sample and 180 uL working solution to each well of a 96-well plate. Mix well.
4. Immediately record the initial absorbance at 450 nm as A1. After 2 min, record the absorbance as A2.
5. Calculate ΔA = A2 - A1.

PDH Activity Calculation

The standard curve is y = 6.7928x - 0.0009, R^2= 0.9991, where y is ΔA and x is the concentration in umol/mL.

1. Calculation Based on Sample Protein Concentration

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that reduces 1 nmol WST-8 per minute per mg of tissue protein.

PDH (nmol/min/mg prot) = (ΔA + 0.0009) ÷ 6.7928 × $V_{total\ reaction}$÷ (V_{sample}× Cpr) ÷ T × 1000 = 736 × ΔA ÷ Cpr

2. Calculation Based on Sample Fresh Weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that reduces 1 nmol WST-8 per minute per g of tissue fresh weight.

PDH (nmol/min/g fresh weight) = (ΔA + 0.0009) ÷ 6.7928 × $V_{reaction\ total}$÷ (W × V_{sample}÷ $V_{sample\ total}$) ÷ T × 1000 = 743 × ΔA ÷ W

3. Calculation Based on Bacterial or Cell Density

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that reduces 1 nmol WST-8 per minute per 10^4bacteria or cells.

PDH (nmol/min/10^4cells) = (ΔA + 0.0009) ÷ 6.7928 × $V_{reaction\ total}$÷ (500 × V_{sample}÷ $V_{sample\ total}$) ÷ T × 1000 = 1.49 × ΔA

Calculation Parameters

$V_{reaction\ total}$	Total volume of the reaction system, 0.2 mL
V_{sample}	Volume of sample added, 0.02 mL
$V_{sample\ total}$	Volume of extraction solution added, 1.01 mL
T	Reaction time, 2 min
Cpr	Sample protein concentration, mg/mL
W	Sample mass, g
500	Total number of bacteria or cells, 500 ten thousand
1000	Conversion factor from umol to nmol

Precautions

1. Before the formal assay, select 2-3 samples with large expected differences for preliminary testing.

2. Required instruments and supplies are not included: microplate reader, water bath, benchtop centrifuge, adjustable pipettes, 96-well plate, mortar, ice, and distilled water.
3. This kit can assay 96 samples.

Visual Reference