Pyruvate Dehydrogenase Assay Kit

Catalog No.: abx298837

Size: 100 Assays

Storage: Store all components at -20°C in the dark.

Application: For quantitative detection of Pyruvate Dehydrogenase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 0.28 U/L – 168.91 U/L

Introduction: Pyruvate dehydrogenase (PDH) is an enzyme that catalyzes the reaction of pyruvate, co-enzyme A (CoA) and NAD^+ to acetyl-CoA, carbon dioxide and NADH. It also links the tricarboxylic acid and glycolysis pathways. Pyruvate dehydrogenase is inhibited by phosphorylation and activated by dephosphorylation. Mutations in or deficiencies of the enzyme have been linked to diseases such as lactic acidosis, neurologic dysfunctions, Leigh syndrome, and oncogene-induced senescence. Measuring pyruvate dehydrogenase activity can provide insight into metabolic functions and oncogenesis.

Abbexa's Pyruvate Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Pyruvate Dehydrogenase activity in a variety of samples. The concentration of the enzyme-catalyzed reaction products can be calculated by measuring the absorbance at 450 nm, from which the enzyme activity can be calculated.

Kit components

1. 96 well microplate
2. Extraction Buffer: 2 × 50 ml
3. Assay Buffer: 40 ml
4. Substrate A: 2 vials
5. Substrate B: 2 vials
6. Dye Reagent: 6 ml
7. Standard: 2 vials

Materials Required But Not Provided

1. Microplate reader (450 nm) and incubator
2. Centrifuge and microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Distilled water
5. Timer
6. Ice
7. Sonicator
8. Mortar

Protocol

A. Preparation of Sample and Reagents

1. Reagents

Bring all reagents to room temperature before use.

- **Substrate A Solution**
  Add 0.5 ml of distilled water into the Substrate A vial and mix thoroughly. Keep on ice in the dark, and ensure that Substrate A has completely dissolved prior to use. The prepared solution can be stored at -20°C in the dark for up to 3 days. Avoid repeated freeze-thaw.

- **Substrate B Solution**
  Add 1.6 ml of distilled water into the Substrate B vial and mix thoroughly. Keep on ice in the dark, and ensure that Substrate B has completely dissolved prior to use. The prepared solution can be stored at -20°C in the dark for up to 7 days. Avoid repeated freeze-thaw.

- **Assay Buffer Working Solution**
  Dilute the Substrate A Solution 101-fold with Assay Buffer Solution (for example, add 50 ml of Assay Buffer to 0.5 ml of Substrate A solution). Prepare immediately before use.

- **0.5 mmol/L Standard Solution**
  Add 1 ml of distilled water into the Standard vial and mix thoroughly to prepare the Standard Solution (concentration 0.5 mmol/L). Ensure that the Standard has completely dissolved prior to use. The prepared solution can be stored at -20°C in the dark for up to 7 days. Avoid repeated freeze-thaw.

2. Sample

- **Serum and Plasma samples**
  Add 0.9 ml of Assay Buffer 1 to 0.1 ml of serum or plasma. Mix thoroughly, then centrifuge at 10,000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Extraction Buffer on ice. Centrifuge at 10,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. The protein concentration of the supernatant should be determined separately.

- **Cell lysates**
  Collect cells and wash with PBS twice. Centrifuge at 1000 × g at 4°C for 10 minutes. Discard the supernatant and keep the pellet. For every 1,000,000 cells (1×10^6), add 200 µl of Extraction Buffer. Sonicate or homogenize manually on ice. Centrifuge at 10000 × g at 4°C for 10 minutes. Take the supernatant for assay immediately. The protein concentration of the supernatant should be determined separately.

B. Assay Procedure

If the expected activity is unknown, a preliminary experiment is recommended to determine if sample dilutions or adjustments to the reaction time are required to bring the measured activity within the detection range of the kit.

1. Label 7 tubes with 0.50 mmol/L, 0.40 mmol/L, 0.30 mmol/L, 0.25 mmol/L, 0.20 mmol/L, 0.10 mmol/L, 0.05 mmol/L, and 0 mmol/L. Prepare the standard curve according to the table below:

Volume of 0.5 mmol/L standard (µl)	Volume of double distilled water (µl)	Standard concentration (mmol/L)
200	0	0.50
160	20	0.40
120	40	0.30
100	80	0.25
80	100	0.20
40	120	0.10
20	160	0.05
0	200	0

2. Set the sample, control and standard wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate. *Each sample requires a control well.*
3. Add 20 µl of sample to the sample and control wells.
4. Add 20 µl of prepared standard to the standard wells.
5. Add 140 µl of Assay Buffer Working Solution to all wells
6. Add 20 µl of Substrate B Solution to the standard and sample wells.
7. Add 20 µl of double distilled water to the standard and sample wells.
8. Add 20 µl of Dye Reagent to all wells.
9. Tap the plate gently to mix and begin the timer.
10. Read and record absorbance at 450 nm after 20 seconds (A_1).
11. Read and record absorbance at 450 nm after 3 minutes 20 seconds (A_2).

C. Calculations

Plot the standard curve with the absorbance value at A_2 as the y values, and the concentration as the x values.
The standard curve is y = ax + b

One Unit (U) of Pyruvate Dehydrogenase activity is defined as the quantity of enzyme required to produce 1 nmol of NADH per minute.

Pyruvate Dehydrogenase activity per g of protein:

$$\textbf{Pyruvate Dehydrogenase}\ (\mathbf{U/g}) = \frac{\Delta \mathbf{A_{Sample}} - \Delta \mathbf{A_{Control}} - \boldsymbol{b}}{\mathbf{a} \times \mathbf{C_{Protein}} \times \mathbf{T}} \times \boldsymbol{f} \times \mathbf{1000}$$

Pyruvate Dehydrogenase activity per L of serum or plasma:

$$\textbf{Pyruvate Dehydrogenase}\ (\mathbf{U/L}) = \frac{\Delta \mathbf{A_{Sample}} - \Delta \mathbf{A_{Control}} - \boldsymbol{b}}{\mathbf{a} \times \mathbf{T}} \times \boldsymbol{f} \times \mathbf{1000}$$

where:

$\Delta \mathbf{A_{Sample}}$	The change in absorbance of the sample well between 20 seconds and 3 minutes 20 seconds $(\mathbf{A_1} - \mathbf{A_2})$
$\Delta \mathbf{A_{Control}}$	The change in absorbance of the control well between 20 seconds and 3 minutes 20 seconds $(\mathbf{A_1} - \mathbf{A_2})$
$\boldsymbol{b}$	The intercept of the standard curve y = ax + b
$\boldsymbol{a}$	The gradient of the standard curve y = ax + b
$\mathbf{T}$	The time of the reaction, 3 minutes
$\boldsymbol{f}$	The dilution factor of the sample prior to testing
$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
1000	Unit conversion (1 mmol = 1000 µmol)