Pyruvate Decarboxylase Assay Kit

Catalog No.: abx298851

Size: 100 Assays

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

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

Detection Range: 0.67 U/L – 27.8 U/L

Sensitivity: 0.67 U/L

Introduction: Pyruvate decarboxylase catalyzes the oxidation of decarboxylation of pyruvate to acetaldehyde. This assay is an indirect method in which the conversion is linked to the activity of the subsequent enzyme alcohol dehydrogenase, which supplied in excess, converts the product acetaldehyde effectively into NAD and ethanol.

Abbexa's Pyruvate Decarboxylase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Pyruvate Decarboxylase activity in various samples. The reaction velocity is proportional to the rate of absorbance resulting from NADH reduction, which can be measured at 340 nm. The PDC activity can then be calculated.

Kit components

1. 96 well microplate
2. Buffer Solution: 20 ml
3. Substrate A: 2 vials
4. Substrate B: 2 vials
5. Enzyme: 2 vials
6. Plate Sealer: 2

Materials Required But Not Provided

1. Microplate reader (340 nm)
2. Microcentrifuge tubes
3. High-precision pipette and sterile pipette tips
4. Normal Saline (0.9% NaCl)
5. Double distilled water
6. Mortar
7. Centrifuge and centrifuge tubes
8. Timer
9. Ice
10. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate A Solution**
  Add 5 ml of Double distilled water into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use. Store on ice in the dark until use. The prepared solution can be stored at -20°C for up to 3 days.

- **Substrate B Solution**
  Add 1.2 ml of Double distilled water into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use. Store on ice in the dark until use. The prepared solution can be stored at -20°C for up to 3 days.

- **Enzyme Solution**
  Add 1.2 ml of Double distilled water to the Enzyme vial and mix thoroughly to prepare the Enzyme Solution. Ensure that the Enzyme has completely dissolved prior to use. Store on ice in the dark until use. The prepared solution can be stored at -20°C for up to 3 days.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 200 µl of Normal Saline (0.9% NaCl) for every 1,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 12,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately. The protein concentration in the supernatant should be determined separately

- **Tissue samples**
  Homogenize 0.1 g of sample in 0.9 ml of Buffer Solution. Centrifuge at 12,000 × g at 4°C for 20 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Serum and Plasma samples**
  Serum and plasma samples can be used directly.

  The following dilution factors are recommended for reference only. The diluent is Normal Saline (0.9% NaCl)

Sample	Dilution factor
Rat serum	1
Rat plasma	1
HL-60 cell sample	3-5
10% Mouse heart tissue homogenate	25-50
10% Mouse kidney tissue homogenate	25-50
10% Mouse Liver tissue homogenate	25-50

B. Assay Procedure

Bring all reagents to room temperature prior to use.

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. Set the sample and blank wells on the 96 well microplate and record their positions. We recommend setting up each standard and sample in duplicate.
2. Add 20 µl of Double distilled water to the blank wells.
3. Add 20 µl of sample to the sample wells.
4. Add 120 µl of Buffer Solution to all wells.
5. Add 20 µl of Enzyme Solution to all wells.
6. Add 20 µl of Substrate Solution B to all wells.
7. Add 20 µl of Substrate Solution A to all wells.
8. Tap the plate gently to mix. Start the timer, then read and record the absorbance at 340 nm after 60 seconds and 180 seconds.

C. Calculations

One unit of Pyruvate Decarboxylase (PDC) activity is defined as the amount of enzyme required to decompose 1 nmol of NADH per minute.

Pyruvate Decarboxylase activity per g of protein:

$$\mathbf{PDC\ (U/g)} = \frac{\boldsymbol{f} \times \mathbf{10^6}}{\mathbf{C_{Protein}} \times \mathbf{T}} \times \frac{\mathbf{\Delta A_{Sample}} - \mathbf{\Delta A_{Blank}}}{\boldsymbol{\varepsilon} \times \boldsymbol{d}}$$

Pyruvate Decarboxylase activity per L of sample:

$$\mathbf{PDC\ (U/L)} = \frac{\boldsymbol{f} \times \mathbf{10^6}}{\mathbf{T}} \times \frac{\mathbf{\Delta A_{Sample}} - \mathbf{\Delta A_{Blank}}}{\boldsymbol{\varepsilon} \times \boldsymbol{d}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in g/L)
$\mathbf{T}$	Reaction time (2 minutes)
$\mathbf{\Delta A_{Sample}}$	$\mathbf{OD_{Sample\ (180\ s)}} - \mathbf{OD_{Sample\ (60\ s)}}$
$\mathbf{\Delta A_{Blank}}$	$\mathbf{OD_{Blank\ (180\ s)}} - \mathbf{OD_{Blank\ (60\ s)}}$
$\times\ \mathbf{10^6}$	Unit conversion: 1 mol = $\mathbf{1} \times \mathbf{10^6}$ µmol
$\boldsymbol{f}$	Sample dilution factor
$\boldsymbol{\varepsilon}$	Molar extinction coefficient of NADH, 6.22×10^3 L/molcm
$\boldsymbol{d}$	Optical path of the microplate, 0.6 cm