QuantiChrom™ Pyruvate Dehydrogenase Assay Kit (DPDH-100)

Quantitative Colorimetric Kinetic Pyruvate Dehydrogenase Activity Determination

DESCRIPTION

PYRUVATE DEHYDROGENASE (PDH) (E.C. 1.2.4.1) is an oxidoreductase which catalyzes the decarboxylation of pyruvate to form acetylated dihydrolipoamide in a thiamine pyrophosphate (TPP)-dependent reaction. PDH is the E1 component of the PDH complex that ultimately produces acetyl-CoA. The PDH complex connects Glycolysis to the Citric Acid Cycle. This non-radioactive colorimetric PDH assay is based on the reduction of the tetrazolium salt MTT in a Phenazine Methosulfate (PMS) coupled reaction to a reduced form of MTT which exhibits an absorption maximum at 565 nm. The intensity of the purple color formed is directly proportional to the PDH activity.

KEY FEATURES

High sensitivity and wide linear range. The detection limit is 0.87 U/L, with linearity up to at least 278 U/L PDH in a 96-well plate assay.

Homogeneous and simple procedure. Simple "mix-and-measure" procedure allows reliable quantitation of PDH activity within 20 minutes.

Robust and amenable to HTS. All reagents are compatible with high-throughput liquid handling instruments.

APPLICATIONS

Direct Assays: PDH activity in biological samples.

Drug Discovery: screening and evaluation of PDH modulators.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	8.0 mL	**TPP Solution:**	500 µL
PMS Solution:	500 µL	**Pyruvate:**	500 µL
MTT Solution:	1.0 mL	**Calibrator:**	1.0 mL

Storage conditions. The kit is shipped at room temperature. Store all components at -20°C upon receipt. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Safety Data Sheet for detailed information.

PROCEDURES

This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at room temperature (25°C) or 37°C.

Sample Preparation:

This assay was optimized using purified PDH obtained from porcine heart (Sigma P7032). For unknown samples, it is recommended that the user try different dilutions of their enzyme preparation to obtain optimal results.

Tissue: Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue in 5 mL buffer containing 100 mM potassium phosphate (pH 7.0) and 1 mM EDTA, per gram of tissue. Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; instead, use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 100 mM potassium phosphate (pH 7.0) and 1 mM EDTA. Centrifuge at 10,000 × g for 15 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation: Equilibrate reagents to desired reaction temperature (e.g. 25°C or 37°C). Briefly centrifuge reagent tubes before use.

Assay Procedure:

1. Transfer 100 µL H_2O (OD_{H2O}) and 100 µL Calibrator (OD_{CAL}) solution into separate wells of a clear, flat-bottom 96-well plate.
2. Transfer 20 µL of each sample into separate wells and 20 µL of Assay or sample buffer to a separate well, as the "no enzyme" blank.

   Prepare sufficient Working Reagent (WR) for all sample wells, including the "no enzyme" blank well, by mixing for each well: 10 µL MTT Solution, 5 µL PMS Solution, 5 µL TPP solution, 5 µL Pyruvate substrate, and 60 µL Assay Buffer. Fresh reconstitution is required. The WR should be made just prior to use and protected from light.

   Add 80 µL of fresh WR to each sample well and the "no enzyme" blank well. Tap plate briefly to mix.
3. Read OD_{565nm} immediately (OD_{S0}), and again after 20 min (OD_{S20}) on a plate reader. (Protect the plate from the light during the incubation.)

CALCULATION

PDH activity can be calculated as follows:

$$\text{PDH Activity} = \frac{\Delta OD_S - \Delta OD_B}{\varepsilon_{mtt} \bullet l} \times \frac{\text{Reaction Vol (µL)}}{t\,\text{(min)} \bullet \text{Sample Vol (µL)}} \times n$$

$$= \frac{\Delta OD_S - \Delta OD_B}{OD_{CAL} - OD_{H20}} \times \frac{273}{t\,\text{(min)}} \times n \quad \textit{(U/L)}$$

where ΔOD_S is the value of the sample at 0 min (OD_{S0}) subtracted from the value of the sample at 20 min (OD_{S20}), and ΔOD_B is the value of the "no enzyme" blank at 0 min (OD_{B0}) subtracted from the value at 20 min (OD_{B20}). OD_{CAL} and OD_{H20} are OD_{565nm} values of the Calibrator and water, respectively, at 20 minutes. Reaction Vol and Sample Vol are 100 µL and 20 µL, respectively. *n* is the dilution factor.

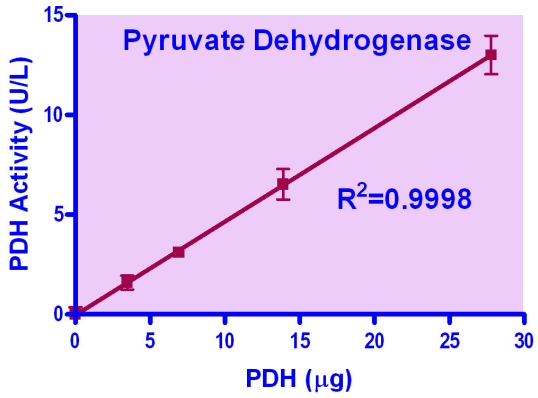
Note: if sample PDH activity exceeds 278 U/L, dilute samples in water and repeat the assay.

Unit definition: 1 Unit (U) of PDH will catalyze the conversion of 1 µmole of pyruvate to acetylated dihydrolipoamide per min at pH 7.4.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat bottom 96-well plates (e.g. e.g. VWR cat# 82050-760) or cuvettes, and plate reader or spectrophotometer capable of measuring OD 565 nm.

EXAMPLES



Titration of porcine heart PDH activity in 96-well plate assay

LITERATURE

1. Gray, LR et al (2014) Regulation of pyruvate metabolism and human disease. Cell. Mol. Life. Sci. 71:2577-2604.
2. Ke, C-J et al (2014). A new spectrophotometric assay for measuring pyruvate dehydrogenase complex activity: a comparative evaluation. Anal. Methods. 6:6381-6388.
3. Sun, W et al (2015) The role of Pyruvate Dehydrogenase Complex in cardiovascular diseases. Life Sci. 121:97-103.