Pyruvate dehydrogenase Assay (PDH)

Cat. No. 8658
100 Tests in 96-well plate

Introduction

Pyruvate dehydrogenase (PDH) is the first component enzyme of the pyruvate dehydrogenase complex (PDC). As a key regulator of tricarboxylic acid (TCA) cycle flux, the PDC catalyzes the conversion of pyruvate into acetyl-CoA and regulates the entry of glucose-carbons into the TCA cycle. PDC deficiency is one of the most common neurodegenerative disorders associated with abnormal mitochondrial metabolism. This colorimetric assay is based on pyruvate dehydrogenase-catalyzed oxidation of pyruvate, where the resulting NADH can then convert a nearly colorless probe to a colored product. The intensity of the colored product is proportional to the amount of PDH in the sample, exhibiting maximum absorbance at 440nm.

Kit Components

Cat. No.	# of vials	Reagent	Quantity	Storage
8658a	1	Assay buffer	25 mL	4°C
8658b	1	PDH positive control	20 µL	-20°C
8658c	1	Developer (10X)	0.1 mL	-20°C
8658d	1	NAD	2.0 mL	-20°C
8658e	1	WST	3.91 mg	-20°C
8658f	1	Substrate	1.6 mL	-20°C
8658g	1	Cofactor	1.7 mL	4°C

Product Use

The PDH Assay kit measures the PDH activity of different types of samples, such as tissues and cell lysate. This product is for research purposes only and not for use in animals, humans, or diagnostic procedures.

Quality Control

Diluted PDH positive control is measured with the PDH Assay kit after different reaction times (Figure 1 and 2). The detection limitation is from 0.005 to 0.08 U/mL.

Shipping

Shipped on dry ice.

Reagents and Positive Control Preparation

- Diluted PDH positive control: Add 1 µl of PDH positive control into 9 µl assay buffer (8658a). Prepare diluted PDH positive control to a final volume of 10 µL/well in a 96-well flat bottom plate.
- Developer solution (1X): dilute developer (10X) (8658c) in assay buffer (8658a) (1:10).

- WST solution: reconstitute each vial of WST with 0.6 mL assay buffer (8658a). Vortex briefly and keep in the dark at -20°C until use. For longer storage, we suggest that you aliquot and store the reconstituted WST solution at -20°C, avoid repeated freeze/thaw cycles.

Procedure (96-well plate)

A. Preparation of test samples and blank

- Cells or tissues can be homogenized in 4 volumes of the assay buffer (8658a). Centrifuge the samples at 10,000 ×g for 10 minutes at 4ºC to remove insoluble material. The soluble fraction may be assayed directly.
- Samples should be serially diluted to make sure the readings are within the detection limitation range. Prepare test samples to a final volume of 10 µL/well in a 96-well flat bottom plate.
- Prepare a blank by adding 10 µL assay buffer (8658a) into one well of the 96-well flat bottom plate.

B. Working reagent preparation and measurements

- Prepare appropriate volume of PDH assay working reagent based on the number of samples to be measured. For each well of reaction, prepare working reagent by mixing 22 µL assay buffer (8658a), 10 µL developer solution (1X), 20 µL NAD (8658d), 5 µL WST solution, 16 µL substrate (8658f), and 17 µL cofactor (8658g).
- Add 90 µL of working reagent mix into each well of the 96-well plate containing the diluted PDH positive control, samples, and blank. Mix well immediately and start recording OD_{440nm} over 30 minute intervals, collecting data every 5 min. Figure 1 shows the data of diluted PDH positive control.

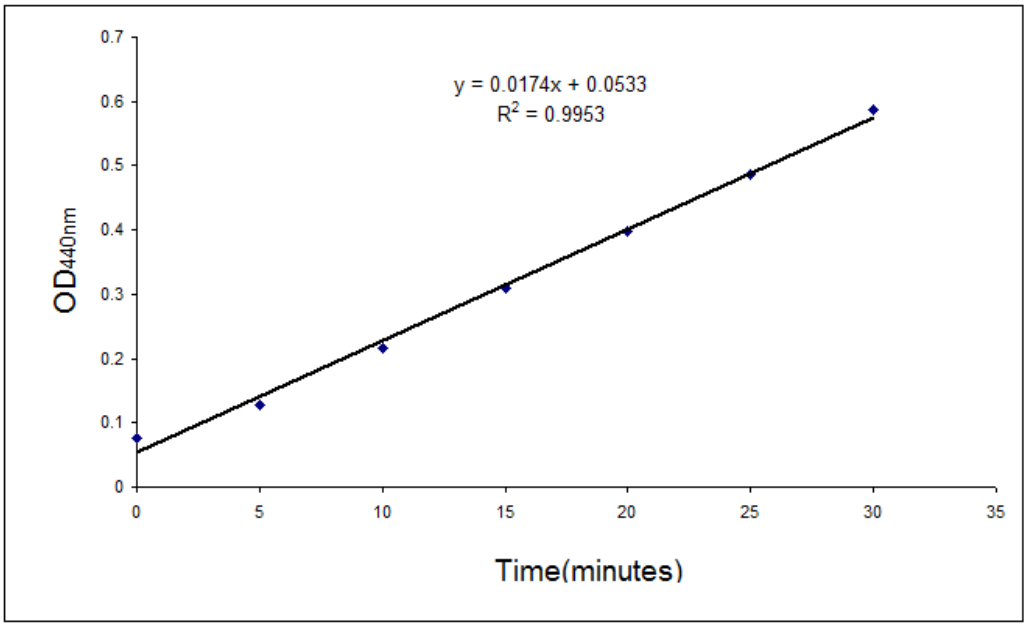

Figure1. Absorbance change of diluted PDH positive control at 440nm.

C. Calculations

- Determine the change in absorbance ΔOD_{440nm}/min by plotting the absorbance value at ΔOD_{440nm} as a function of reaction time to obtain the slope of the linear portion of the curve, as shown in Figure 2.

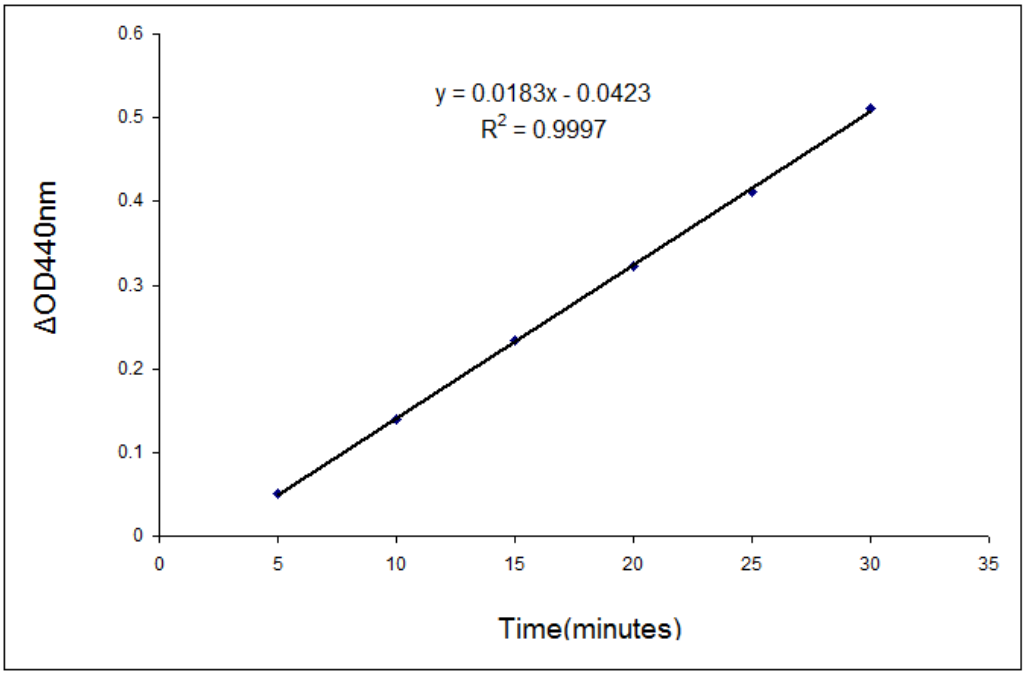

Figure 2. The change in absorbance ΔOD_{440nm} of diluted PDH positive control during the time at 440nm.

- Calculate PDH activity using the following formula:

$$\text{PDH (U/ml)} = \frac{(\Delta OD_{440nm}/\text{min-blank}) \times 100\ \mu l}{11.53\ mM^{-1} \times 10\ \mu l} \times \text{sample dilution}$$

Note: The actual extinction coefficient of the formed WST-1 formazan at 440nm is 37 $mM^{-1}cm^{-1}$. This value has been adjusted for the path length of the solution in the 96-well plate.

Unit definition: One unit makes 1.0 µmol of WST-1 to WST-1 formazan per minute at pH 7.4 at 25 ºC

- Use the formula to calculate PDH positive control activity:

$$\text{PDH positive control (U/ml)} = \frac{(0.0183\text{-}0.0024) \times 100\ \mu l}{11.53\ mM^{-1} \times 10\ \mu l} \text{ X } 10 = 0.14\ \text{(U/ml)}$$