MANUAL

Pyruvate Dehydrogenase Activity Assay Kit

Catalog #: MA-PDH

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ISO 13485:2016

Introduction

Pyruvate dehydrogenase (PDH) is the first component enzyme of the pyruvate dehydrogenase complex (PDC). It catalyzes the conversion of pyruvate to acetyl-CoA and CO_2, thereby linking the tricarboxylic acid (TCA) and glycolysis pathways. Mutations in PDH have been linked to pyruvate dehydrogenase deficiency (causing lactic acidosis and neurologic dysfunctions) and Leigh syndrome. PDH has also been implicated in oncogene-induced senescence. Measuring PDH activity can provide insights into metabolic functions and oncogenesis

Pyruvate Dehydrogenase Activity Assay Kit provides a simple, reproducible, and sensitive tool for measuring pyruvate dehydrogenase activity in cell lysates, plasma, serum, urine, and other biological liquid samples. This assay features low disturbance and high sensitivity. It 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.

Storage

The entire kit should be stored below -20 °C for up to 6 months from the date of shipment. For prepared reagent storage, see table below.

Component	Size / Description	Storage After Preparation
Microplate	A 96-well (12 strips x 8 wells) plate	RT*
Assay Buffer	20 ml	2–8 °C, 3 months
Pyruvate Dehydrogenase Positive Control	1 vial	Store at -20 °C, 3 months
PMS	1 vial (Lyophilized)	Prepare aliquots, store at -20 °C. Avoid multiple freeze-thaw cycles. Protect from light.
MTT	3 vials (Lyophilized)	Prepare aliquots, store at -20 °C. Avoid multiple freeze-thaw cycles. Protect from light.
Cofactor	1 vial (Lyophilized)	Prepare aliquots, store at -20 °C. Avoid multiple freeze-thaw cycles.
Pyruvate	1 vial	2–8 °C, 3 months

**RT = room temperature*

Additional Materials Required

1. Microplate reader capable of measuring absorbance at 566 nm
2. Precision pipettes to deliver 2 µl to 1 ml volumes
3. Multi-channel pipettes to deliver 20 µl to 200 µl volumes
4. Tubes to prepare sample dilutions
5. 50 ml conical tubes
6. Ultrapure water

Sample Tips and General Considerations

NOTE: Optimal methods of sample preparation will need to be determined by each researcher empirically based on researched literature and knowledge of the samples.

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid sonication of 1 ml or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.

1. Cell lysates can be prepared as follows:

For attached cells, remove supernatant from cell culture, wash cells twice with cold 1X PBS (for suspension cells, and pellet the cells by spinning down the cells at 1,000 x g for 10 min) making sure to remove any remaining PBS before adding lysis buffer. Solubilize the cells at $2x10^7$ cells/ml in lysis buffer containing protease inhibitors. Pipette up and down to resuspend cells and rock the lysates gently at 2–8 °C for 30 minutes. Transfer extracts to microfuge tubes and centrifuge at 14,000 x g for 10 minutes.
It is recommended that sample protein concentrations should be determined using a total protein assay. Lysates should be used immediately or aliquot and stored at -70 °C. Thawed lysates should be kept on ice prior to use.
General tips for preparing lysate samples can be viewed on the online Resources page of the website: https://www.raybiotech.com/tips-on-sample-preparation/

2. Plasma samples:

Collect blood with an anticoagulant such as citrate, EDTA or oxalate and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Collect plasma supernatant without disturbing the white buffy layer. Sample should be tested immediately or frozen at -80°C for storage. The sample may be assayed directly.

3. Serum samples:

Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Samples should be tested immediately or frozen at -80°C for storage. The sample may be assayed directly.

4. Urine and other biological liquid:

To remove insoluble particles, spin at 10,000 x g for 5 min. The supernatant may be assayed directly.

NOTE:

1. *When analyzing PDH activity from mitochondria, it is recommended to isolate the mitochondria from fresh tissue or cells.*
2. *Optimal experimental conditions for samples must be determined by the investigator. A set of serial*

dilutions is recommended for unknown samples to achieve optimal assay results.

Reagent Preparation

A. Working Solution

1. PMS: Briefly spin down the vial and add 250 µL ultrapure water to the vial. Mix well. Keep on ice.
2. MTT: There are three vials in total. Briefly spin down each vial and add 125 µL ultrapure water to each vial. Mix well. Keep on ice.
3. Cofactor: Briefly spin down the vial and add 250 µL ultrapure water to the vial. Mix well. Keep on ice.
4. **Working Solution**: *For each well*, add 2 µL of reconstituted PMS, 3 µL of reconstituted MTT, 2 µL of reconstituted Cofactor, and 2.5 µL of Pyruvate provided into 90.5 µL of assay buffer to make the Working Solution (protect from light). Mix well. Scale up to the number of wells you need **(always make enough for three or four additional wells to account for pipetting error)**.
   - For example, for 50 wells needed, prepare enough Working Solution for 54 wells: add 108 µL of reconstituted PMS, 162 µL of reconstituted MTT, 108 µL of reconstituted Cofactor, and 135 µL of Pyruvate provided into 4887 µL of Assay Buffer to make the Working Solution. Keep on ice.

Working Solution (Protect from light)		
Number of wells	**1 well**	**50 wells (additional added for pipetting error)**
Assay Buffer	90.5 µl	4887 µl
PMS	2 µl	108 µl
MTT	3 µl	162 µl
Cofactor	2 µl	108 µl
Pyruvate	2.5 µl	135 µl

B. Positive Control

Pyruvate Dehydrogenase (PDH) Positive Control: Take 13 µL from the Pyruvate Dehydrogenase Positive Control tube and add it to 30 µL Assay Buffer. Mix well. This is the PDH Positive Control. Keep on ice.

Assay Procedure

Positive control, samples, and Assay Buffer (used as a blank) should be assayed in duplicate or triplicate. A freshly prepared positive control should be used each time the assay is performed.

1. Prepare Working Solution (See Reagent Preparation, section A).
2. Prepare Positive Control (See Reagent Preparation, section B).
3. Add 20 µL of the diluted PDH Positive Control, Assay Buffer (used as a blank), or unknown samples to the 96-well microtiter plate.
4. Add 100 µL of Working Solution to each well. Mix thoroughly. Read and record absorbance at 566nm every minute for 3 time points at room temperature (25 ^{o}C). These will be readings A1, A2, A3.

Calculation of Results

Calculate the mean absorbance for each set of duplicate/triplicate samples, Positive Control, and Assay Buffer blank.

Calculate the average absorbance difference per minute ΔOD_{566nm}/min.

$$\Delta OD_{566nm}/min = \frac{(A1 - A2) + (A2 - A3)}{2}$$

Subtract the absorbance difference per minute of the Blank wells from the absorbance difference per minute of the samples and Positive Control. This is the corrected absorbance difference per minute.

$$\Delta OD'_{566nm}/min = \Delta OD_{566nm}/min - \Delta OD^{Blank}_{566nm}/min$$

One international Unit (U/L) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per minute.

$$\text{PDH activity (U/L)} = \frac{\Delta OD'_{566nm}/min \times 120\ \mu l \times 1000}{6.23\ mM^{-1} \times 20\ \mu l} \times \text{sample dilution factor}$$

Where: $\Delta OD'_{566nm}$ /min = Corrected absorbance change per minute

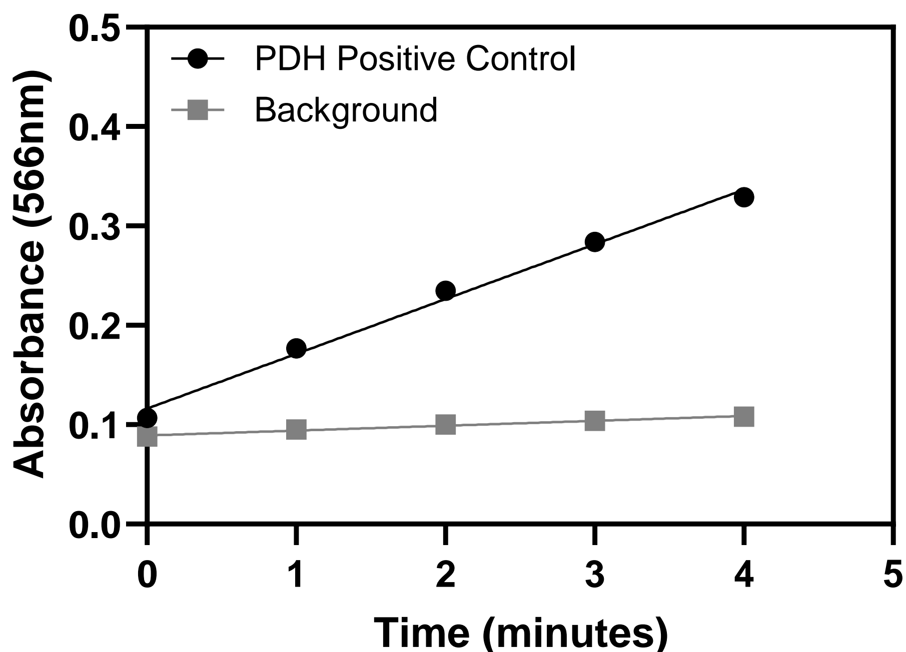
1000 = Conversion of U/ml to U/L

6.23 mM^{-1} is the adjusted extinction coefficient for the reduced MTT at 566nm with the current path length of the solution in a 96-well plate.

*PDH activity International System of Units (SI Units) (nkat/L) = PDH activity (U/L)*16.67*

Note: If the calculated PDH activity of the sample is higher than 100 U/L, dilute the sample in Assay Buffer and repeat the assay.

A. Typical Data



Example of the absorbance value of Pyruvate Dehydrogenase (PDH) Positive Control versus Time. This is an example. Results will vary.

B. Linear Range

0.38 U/L to 100 U/L PDH activity.

C. Reproducibility

Intra-assay Precision (Precision within an assay):
To assess intra-assay precision, 16 wells per sample (total of 4 samples) were tested on a single plate. The intra-assay coefficient of variation was found to be 5.0%.

Inter-assay Precision (Precision between assays):
To assess inter-assay precision, 4 samples were tested in separate assays (n=4). The inter-assay coefficient of variation was found to be 6.5%.

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