# Product Information

## Pyruvate Dehydrogenase (PDH) Activity Colorimetric Assay Kit

### I. Kit Contents:

| Components | K2218-96<br>96 assays | Cap Color |
|---|---|---|
| PDH Assay Buffer | 25 ml | WM |
| PDH Substrate (Lyophilized) | 1 vial | Blue |
| PDH Developer (Lyophilized) | 1 vial | Red |
| NADH Standard (Lyophilized) | 1 vial | Yellow |
| PDH Positive Control | 10 µl | Orange |

### II. Introduction:

Pyruvate Dehydrogenase (PDH) plays an important role in carbohydrate metabolism. Combining with dihydrolipoyl transacetylase (E2) and dihydrolipoyl dehydrogenase (E3), PDH forms a well-characterized enzyme complex. In the presence of $NAD^+$ and CoA, PDH converts pyruvate into acetyl-CoA. PDH also links glycolysis metabolic pathway to the citric acid cycle. PDH activity is inhibited by high ratios of intracellular Acetyl-CoA/CoA, NADH/$NAD^+$ or ATP/ADP. In humans, PDH deficiency inhibits mitochondrial function and is involved in neurodegenerative diseases. PDH deficiency is X-linked and results in 2 forms of abnormality: seizure and/or neuropathological spasm (a neurological form) and lactic acidosis (a metabolic form). PDH is also a target of oncogene-induced senescence. Activation of PDH can increase respiration and redox stress, and enhance pyruvate utilization.

The Pyruvate Dehydrogenase (PDH) Activity Colorimetric Assay Kit provides a sensitive, simple, fast and convenient way for detection PDH activity in various samples based on colorimetric method. In the assay, PDH changes pyruvate to an intermediate which reduces the developer to a colored (450 nm) product. The kit can detect PDH activity lower than 0.1 mU in a variety of samples.

### III. Application:

Measurement of pyruvate dehydrogenase activity in various tissues/cells.

Analysis of cell signaling pathway.

### III. Sample Type:

Animal tissues: heart, liver, muscle, etc.

Purified mitochondria.

Cell culture: Adherent or suspension cells.

### V. User Supplied Reagents and Equipment:

96-well clear plate with flat bottom.

Multi-well spectrophotometer (ELISA reader).

### VI. Storage and Handling:

Store kit at -20℃, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

## VII. Reagent Preparation and Storage Conditions:

PDH Assay Buffer: Warm to room temperature before use. Store at either 4℃ or -20℃.

PDH Substrate: Reconstitute with 220 µl $dH_2O$. Store at -20℃. Keep on ice while in use. Use within two months.

PDH Developer: Reconstitute with 220 µl $dH_2O$. Gently pipette up and down to dissolve completely. Store at -20℃. Use within two months.

NADH Standard: Reconstitute with 400 µl $dH_2O$ to generate 1.25 mM NADH Standard solution. Aliquot and store at –20℃. Keep on ice while in use. Use within two months.

PDH Positive Control: Add 100 µl PDH Assay Buffer to the Positive Control and mix thoroughly. Aliquot and store at –20℃. Keep on ice while in use. Use within two months.

## VIII. Pyruvate Dehydrogenase Assay Protocol:

1. Sample Preparation: Rapidly homogenize tissue (10 mg) or cells (1 x $10^6$) with 100 µl ice cold PDH Assay Buffer, and keep on ice for 10 min. Centrifuge at 10,000 x g for 5 min. and transfer the supernatant to a fresh tube. Add 5 - 50 µl sample per well & adjust the volume to 50 µl with PDH Assay Buffer. To check PDH activity in mitochondria, isolate the mitochondria from fresh tissue or cells using Mitochondria Isolation Kit for Tissue and Cultured Cells. Add 5 - 50 µl of isolated mitochondria per well, adjust the volume to 50 µl with PDH Assay Buffer. For the PDH positive control, take 1 - 10 µl of PDH Positive Control into desired well(s) and adjust the final volume to 50 µl with PDH Assay Buffer.

Note:

a. For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

b. For samples exhibiting significant background, prepare parallel sample well(s) as background controls.

c. Small molecules in some tissues such as liver may interfere with the assay. To remove small molecules, we suggest using an ammonium sulfate method. Pipette 50 - 100 µl of lysate into a fresh tube, add 2X volume of saturated ammonium sulfate ( ~ 4.1 M at room temperature) and keep on ice for 20 min. Spin down at 10,000 X g for 5 min., carefully remove and discard the supernatant, and resuspend the pellet to the original volume with PDH Assay Buffer.

2. NADH Standard Curve: Add 0, 2, 4, 6, 8 and 10 µl of 1.25 mM NADH Standard into a series of wells in 96 well plate to generate 0, 2.5, 5.0, 7.5, 10 and 12.5 nmol/well of NADH Standard. Adjust the volume to 50 µl/well with PDH Assay Buffer.

3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Mix containing:

| | Reaction Mix | Background Control Mix |
|---|---|---|
| PDH Assay Buffer | 46 µl | 48 µl |
| PDH Developer | 2 µl | 2 µl |
| PDH Substrate | 2 µl | --- |

Mix and add 50 µl of the Reaction Mix to each well containing the Standard, Positive Control and test samples.

For background correction, add 50 µl of Background Control Mix (without substrate) to sample background control well(s) and mix well.

4. Measurement: Measure absorbance immediately at 450 nm in kinetic mode for 10-60 min. at 37℃.

Note: Incubation time depends on the pyruvate dehydrogenase activity in samples. We recommend measuring the OD in kinetic mode, and choosing two time points (T1 & T2) in the linear range to calculate the pyruvate dehydrogenase activity of the samples. The NADH Standard Curve can be read in Endpoint mode (i.e., at the end of the incubation time).

5. Calculation: Subtract 0 Standard reading from all readings. Plot the NADH Standard Curve. If sample background control reading is significant, subtract the background control reading from its paired sample reading. Calculate the pyruvate dehydrogenase activity of the test sample: $\Delta OD = A2 – A1$. Apply the $\Delta OD$ to the NADH Standard Curve to get B nmol of NADH generated during the reaction time ($\Delta T = T2 - T1$).

$$\text{Sample Pyruvate Dehydrogenase Activity} = B/(\Delta T \times V) \times D = \text{nmol/min/ml} = \text{mU/ml}$$

Where: B = NADH amount from Standard Curve (nmol).

$\Delta T$ = reaction time (min.).

V = sample volume added into the reaction well (ml).

D = Dilution Factor.

Unit Definition: One unit of pyruvate dehydrogenase is the amount of enzyme that generates 1.0 μmol of NADH per min. at pH 7.5 at 37℃.

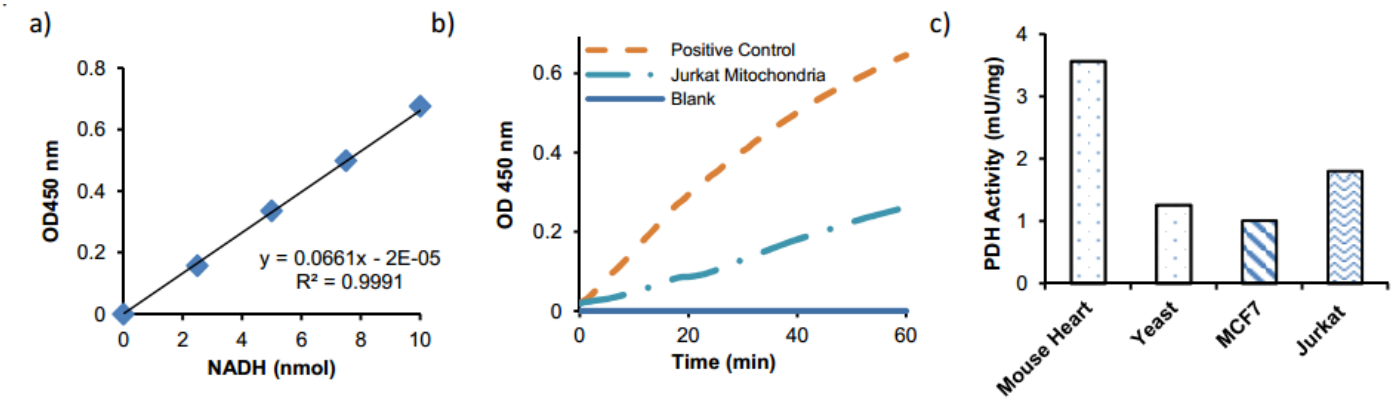


**Figure:** (a) NADH standard curve; (b) Pyruvate Dehydrogenase activity in Jurkat mitochondria (100 μg); (c) Pyruvate Dehydrogenase specific activity was calculated in mitochondria prepared from mouse heart (10 μg), *S. cerevisiae* (10 μg), MCF-7 cells (30 μg) or Jurkat cells (35 μg). Assays were performed following the kit protocol.

## Frequently Asked Questions

1. Can the supernatants from the lysed cellsbe frozen and stored until all the cells are ready to use so that all the samples can be run at the same time?

Although we always prefer fresh samples, the cell lysates can be stored at -80℃ before the assay. The lysates should be aliquoted if the assay will be repeated with the same samples to minimize freezing and thawing. Alternately, all cells can be stored at -80℃ and the lysates can be prepared on the day of the experiment.

2. Is it necessary to purify mitochondria to measure PDGH activity with this assay?

If you use total cell lysate, total PDH activity will be measured. Only if you fractionate the mitochondria and use that as sample, exclusively mitochondrial PDH activity will be measured.

3. On what species will this kit work?

Our kits are desgined and optimized with mammalian samples but this assay should measure PDH activity (pyruvate dehydrogenation is an evolutionarily conserved reaction) in any sample irrespective of species as long as there is PDH activity within the measurable range of the assay.

4. Can frozen samples be used with this assay?

Fresh samples are always preferred over frozen samples. However, frozen samples can also be used, provided, they were frozen right after isolation, were not freeze thawed multiple time (for which we recommend aliquoting the samples before freezing) and have been frozen for relatively short periods.

5. Is it possible to use a different wavelength than recommended for the final analysis?

It is always recommended to use the exact recommended wavelength for the most efficient results. However, most plate readers have flexibility in their band width of detection in increments of +/- 10 nm. Depending on this flexibility range, you can deviate from the recommended wavelengths within limits.

6. What is the exact volume of sample required for this assay?

There is no specific volume we can recommend for the amount any sample to be used since it is completely sample concentration and quality based. It is recommended to do a pilot expt with multiple sample volumes to determine the optimal volume which gives a reading within the linear range of the standard curve. Please refer to the citations for this product to see what other clients have used with similar sample types.

7. What is the shelf life of this kit?

This kit is good for 12 months from the date of shipment in the unopened form when stored at the appropriate temperature and appropriate conditions. After opening and reconstitution, some of the components in this kit are good for 2 months at -20℃. Please refer to the datasheet for storage information and shelf life of each of the components.

8. Why are the standard curve values lower than those shown on the datasheet?

There are multiple factors which influence the signals like the incubation times, room temperature, handling etc. In general, to increase the value of the standards, the incubation time can be increased. As long as the standard curve is linear, it should be fine to use, since all the samples will also be measured under the same conditions on this curve.

**For research use only! Not to be used in humans.**

## Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

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