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Pyruvate Dehydrogenase Activity Colorimetric Assay Kit

12/13

(Catalog # K679-100; 100 assays; Store at -20°C)

I. Introduction:

Pyruvate Dehydrogenase (PDH) (EC 1.2.4.1) has a vital role in carbohydrate metabolism. It forms a well-characterized enzyme complex with dihydrolipoyl transacetylase (E2) and dihydrolipoyl dehydrogenase (E3). PDH converts pyruvate into acetyl-CoA in the presence of NAD and CoA, and links glycolysis to the citric acid cycle. PDH activity is inhibited by high intracellular ratios of ATP/ADP, NADH/NAD or Acetyl-CoA/CoA. In humans, PDH deficiency reduces mitochondrial function and is linked to neurodegenerative diseases. PDH deficiency is X-linked; it results in 2 forms of abnormality: a metabolic form (lactic acidosis) and a neurological form (seizure and/or neuropathological spasm). Recent studies show that PDH is a target of oncogene-induced senescence; activation of PDH enhances pyruvate utilization and increases respiration and redox stress. BioVision's PDH assay kit provides a quick and easy way for monitoring PDH activity in various samples. In the assay, PDH converts pyruvate into an intermediate, which reduces the developer to a colored product with strong absorbance at 450 nm. The assay is simple, sensitive and can detect pyruvate dehydrogenase activity lower than 0.1 mU in a variety of samples.

II. 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

IV. Kit Contents:

Components	K679-100	Cap Code	Part Number
PDH Assay Buffer	25 ml	WM	K679-100-1
PDH Substrate (Lyophilized)	1 vial	Blue	K679-100-2
PDH Developer (Lyophilized)	1 vial	Red	K679-100-3
NADH Standard (Lyophilized)	1 vial	Yellow	K679-100-4
PDH Positive Control	10 µl	Orange	K679-100-5

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°C, 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 4ºC or -20ºC.
- **PDH Substrate:** Reconstitute with 220 µl dH_2O. Store at -20°C. 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°C. 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°C. 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°C. 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 BioVision's Mitochondria Isolation Kit for Tissue and Cultured Cells (K288-50). 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

[Cat. # 7096] 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°C.

Note: Incubation time depends on the pyruvate dehydrogenase activity in samples. We recommend measuring the OD in kinetic mode, and choosing two time points (T_1 & T_2) 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: $\Delta OD = A_2 - A_1$. Apply the ΔOD to the NADH Standard Curve to get B nmol of NADH generated during the reaction time ($\Delta T = T_2 - T_1$).

Sample Pyruvate Dehydrogenase Activity = B/(ΔT X V) x D = nmol/min/ml = mU/ml

Where: **B** = NADH amount from Standard Curve (nmol).
ΔT = 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°C.

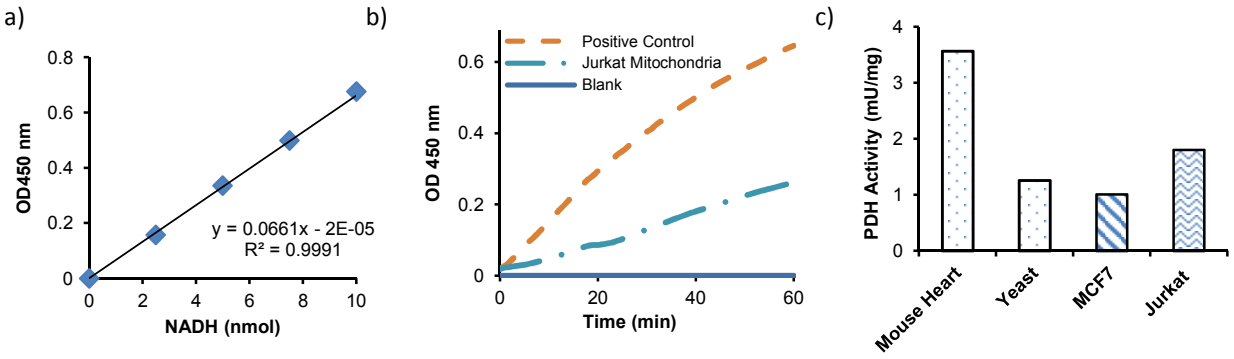

Figure: (a) NADH standard curve; (b) Pyruvate Dehydrogenase activity in Jurkat mitochondria (100 µg); (c) Pyruvate Dehydrogenase specific activity was calculated in mitochondria prepared from mouse heart (10 µg), *S. cerevisiae* (10 µg), MCF-7 cells (30 µg) or Jurkat cells (35 µg). Assays were performed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

IX. RELATED PRODUCTS:

Malate Colorimetric Assay Kit (K637)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
Citrate Colorimetric/Fluorometric Assay Kit (K655)
Citrate Synthase Activity Colorimetric Assay Kit (K318)
Succinate (Succinic Acid) Colorimetric Assay Kit (K649)
α-Ketoglutarate Colorimetric Assay Kit (K677)
Fumarate Colorimetric Assay Kit (K633)
PicoProbe™ Acetyl-CoA Fluorometric Assay Kit (K317)
Oxaloacetate Colorimetric/Fluorometric Assay kit (K659)
Isocitrate Colorimetric Assay Kit (K656)
Isocitrate Dehydrogenase Activity Assay Kit (K756)
Aconitase Activity Colorimetric Assay Kit (K716)

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