



ab109882 –
Pyruvate dehydrogenase
(PDH) Enzyme Activity
Dipstick Assay Kit

Instructions for Use

For the quantitative measurement of Pyruvate dehydrogenase (PDH) activity in samples from human, mouse, rat, bovine and porcine

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

ab109882 is used to quantify the activity of the PDH enzyme complex. This is accomplished by immunocapturing PDH with an anti-PDH antibody immobilized on a defined section of a dipstick. The enzyme complex is immunocaptured in its native form and activity is measured by the reaction scheme as illustrated in Figure 1. PDH activity is visualized by coupling PDH-dependent production of NADH to the reduction of NBT in the presence of excess diaphorase, forming an insoluble intensely colored precipitate at the capture line. The signal intensity is measured by a dipstick reader or analyzed by other imaging systems such as a flatbed scanner.

Purified mitochondria can be used in this assay. However, homogenized tissue and whole cells can also be used without the need for mitochondrial isolation.

Pyruvate dehydrogenase activity is regulated by PDH kinase and PDH phosphatase. Cells grown in glucose media derive most of their energy from glycolysis; therefore, most of their PDH complex may be in an inactive phosphorylated form at the time of isolation. Growing cells in alternate carbon sources such as glutamine/galactose may up-regulate PDH activity. This kit does not include PDH kinases, PDH phosphatases or their respective inhibitors. These may be incorporated into the assay at the user's discretion.

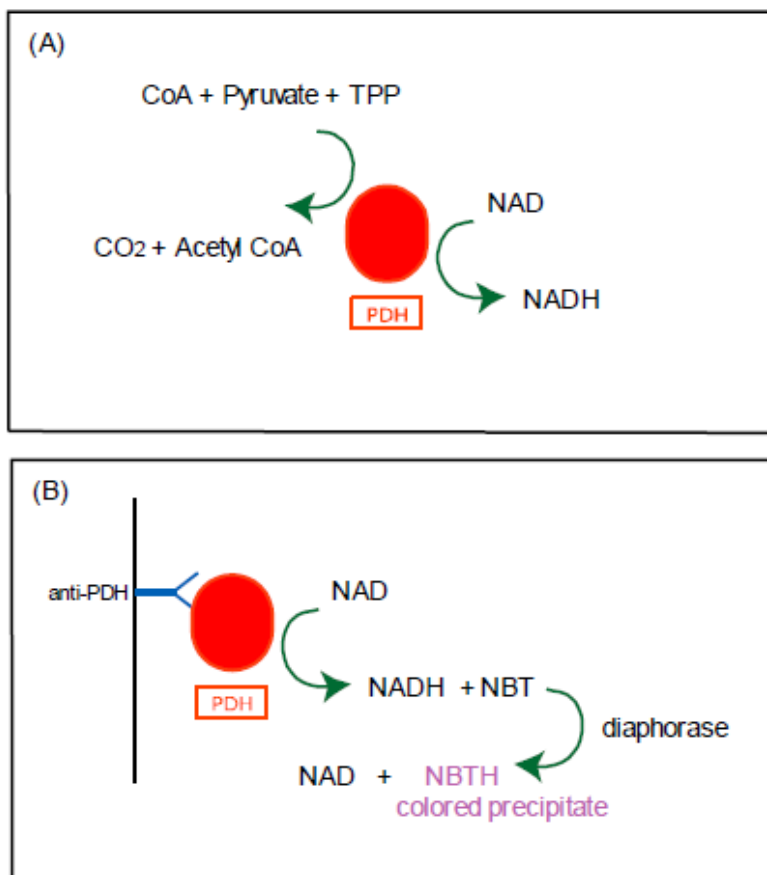
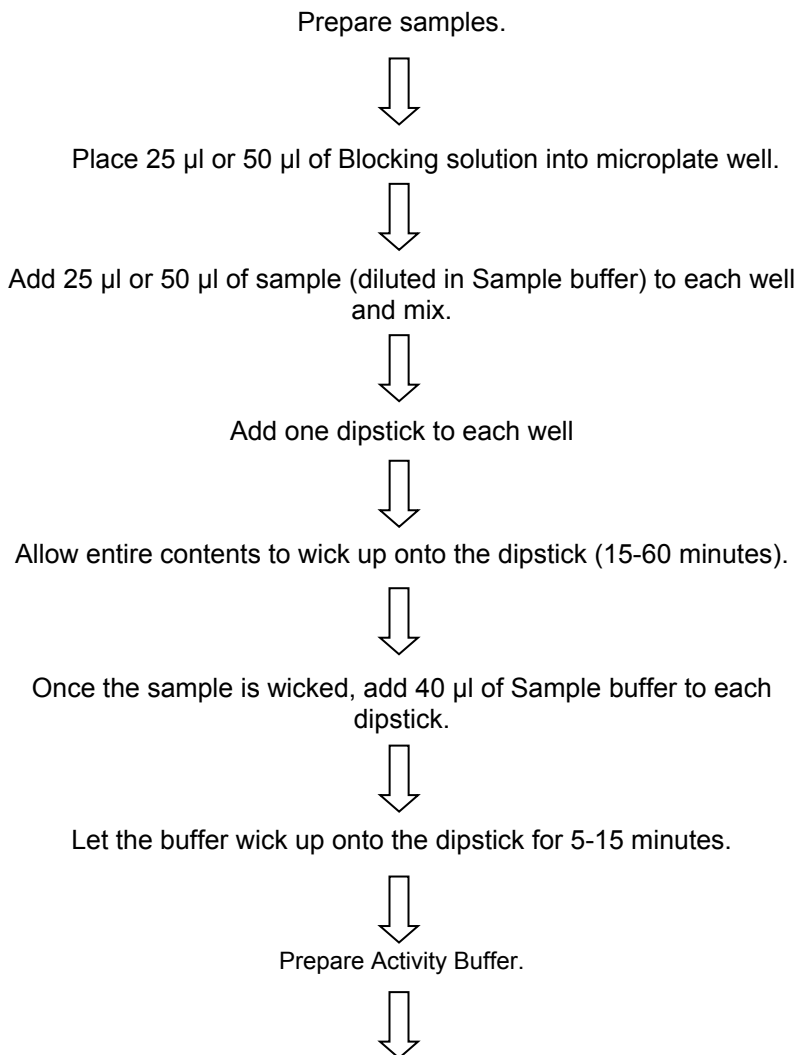


Figure 1: Illustration of the PDH activity reaction. (A) The PDH activity reaction generates NADH. (B) The NADH produced is coupled to the reduction and precipitation of a colored dye.

2. Assay Summary



Add 300 μ l of Activity Buffer for each sample into an empty microplate well.



Remove the wicking pad from the dipstick, especially at the junction with the membrane.



Transfer each dipstick to Activity Buffer containing microplate well and incubate for 40 to 60 minutes.



Add purified water to a new well for each dipstick and wash for 5 minutes.



Dry dipsticks and measure signal.

3. Kit Contents

Sufficient materials for 90 or 30 measurements.

Item	Quantity	
Dipsticks	90	30
Sample Buffer	3 x 30 ml	1 x 30 ml
Blocking solution	3 x 2 ml	1 x 2 ml
Detergent	3 x 1 ml	1 x 1 ml
20X Reagent mix	3 x 0.6 ml	1 x 0.6 ml
Diaphorase	3 x 0.4 ml	1 x 0.4 ml
NBT	3 x 40 μ l	1 x 40 μ l
96-well microplate	6	2

4. Storage and Handling

Store dipsticks at room temperature out of direct sunlight in their provided desiccant container. Avoid high humidity conditions. Store Sample buffer at 4°C. Store 20X Reagent Mix, Detergent, and Diaphorase at -80°C, but these may be aliquoted upon receipt to prevent freeze/thaw cycles. Store Blocking solution at -20°C. Store NBT in the dark at -80°C.

5. Additional Materials Required

- Dipstick reader or other imaging system
- Method for determining protein concentration
- Pipetting devices
- Protease inhibitors

6. Preparation of Samples

The preparation of the sample is a critical step in this procedure. The effective solubilization of the mitochondrial membranes in the sample is necessary. However, if the detergent to membrane protein ratio is too high, PDH may become destabilized. Please choose the sample preparation method that best fits your sample type. Follow each of the protocol steps carefully.

Note: *Samples must be kept on ice.*

A. Sample Preparation Method 1: Standard method for purified mitochondria and muscle or brain tissue homogenates.

1. Pellet the sample by centrifugation.
2. Re-suspend the pellet in Sample buffer to a protein concentration of 5 mg/ml (measure using a standard protein concentration determination method).
3. Add 1/10 volume of supplied detergent.
4. Proceed directly to Dipstick Procedure or freeze samples at -80°C.

B. Sample Preparation Method 2: For soft tissue homogenates that contain low levels of PDH (e.g. liver tissue).

1. Homogenize tissue thoroughly using a micro-tissue grinder or homogenizer. (ab110169/MS851 Mitochondria Isolation Kit available from Abcam).
2. Measure the protein concentration.
3. Adjust protein concentration of the sample to 25 mg/ml in Sample buffer.

4. Add 1/10 volume of the supplied detergent.
5. Proceed directly to Dipstick Procedure or freeze samples at -80°C.

C. Sample Preparation Method 3: For cultured cell extract containing low levels of PDH.

1. Pellet the cells by centrifugation.
2. Re-suspend the pellet in 5 volumes of Sample buffer.
3. Add 1/10 volume of supplied detergent.
4. Keep sample on ice for 10 minutes.
5. Centrifuge sample for 10 minutes at 3,000 rpm.
6. Transfer extracted supernatant to a new tube and determine the protein concentration by a standard method that is detergent compatible.
7. Proceed directly to Dipstick Procedure or freeze samples at -80°C.

7. Dipstick Procedure

The assay is most accurate with a user established standard curve for interpolation of the signal intensity. Following the protein concentration ranges as defined in Table 1, generate a standard curve using a positive control sample.

Sample Preparation Method	Sample Type	Working Range
1	Tissue mitochondria	1 – 10 µg / 25 µl
1	Muscle tissue extract	10 – 100 µg / 25 µl
2	Liver tissue extract	25 – 150 µg / 25 µl
3	Fibroblast extract	40 – 250 µg / 50 µl
3	Hepatocellular carcinoma extract	20 – 160 µg / 50 µl

Table 1. Suggested working range for different sample types. Note: Cultured cells may require increased sample volume to accommodate larger sample load.

1. Dilute sample with appropriate volume of Sample buffer to a protein concentration as per Table 1.
2. Add 25 μ l or 50 μ l of Blocking solution to an empty well of the microplate.
3. To the same well, add 25 μ l or 50 μ l of diluted sample prepared in step 1. Mix by pipetting.
4. Gently add a dipstick to the sample mix in the microplate well. Make sure to place the thin/nitrocellulose end of the dipstick down.
5. Allow the sample to wick up onto the dipstick towards the wicking pad (thick end of dipstick). This takes 12-60 minutes depending on sample volume and viscosity. *The entire sample volume has to be absorbed by the dipstick before proceeding to the next step, but do not allow the dipstick to dry at any time during this procedure.*
6. Now, wash the dipstick by adding 40 μ l of Sample buffer to each microplate well with a dipstick once the sample mix is wicked completely.
7. Allow Sample buffer to wick for 5-15 minutes. *Do not allow the dipstick to dry out.*

8. Prepare the Activity Buffer by combining Sample buffer, 20X Reagent Mix, Diaphorase, and NBT as per Table 2.

No. of Dipsticks	Sample Buffer (ml)	20x Reagent Mix (μ l)	Diaphorase (μ l)	NBT (μ l)
10	3.3 ml	166	133	7
20	6.6 ml	333	266	14
30	10 ml	500	400	20

Table 2: Preparation of Activity Buffer

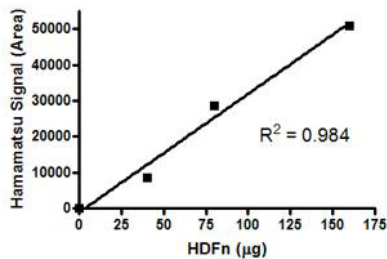
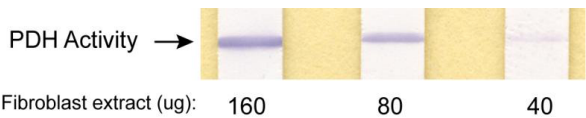
9. Add 300 μ l of Activity Buffer to an empty microplate well for each dipstick.
10. Now, remove the wicking pad from the dipstick. Make sure to remove the pad at the junction with the membrane.
11. Place the dipstick in a well with Activity Buffer. The signal will appear 5-7 mm from the bottom of the dipstick in approximately 10 - 20 minutes
12. Develop for between 40 to 60 minutes. *Since this is an end-point reaction, develop all dipsticks for the same amount of time.*
13. Add 300 μ l deionized water to an empty well of the microplate.
14. After signal development, add the dipstick to the well with deionized water. Wash for 5 minutes.
15. Dry the dipstick and measure the signal intensity with a dipstick reader or other imaging system, e.g. flat-bed scanner.

8. Data Analysis

Below is an example using ab109882 to measure PDH activity in human fibroblast sample. Samples were prepared as described in the Sample Preparation section (Method 3). All data were analyzed using a Dipstick Reader and GraphPad software.

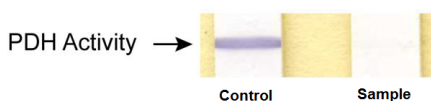
A. Generating a standard curve

Shown are developed dipsticks from a 1:2 dilution series using a positive control sample and the associated standard curve. Starting material was 160 µg of fibroblast protein extract in a 100 µl reaction volume (50 µl sample in Sample buffer plus 50 µl of Blocking solution). The dipsticks were developed for 1 hour in Activity buffer.



B. Analysis of samples

Based on the standard curve, 160 µg of protein extract were loaded onto a dipstick for each sample. The figure below shows two developed dipsticks, a control sample and a sample with a mutation in the E1α subunit of PDH. The analysis of the signal intensity and interpolation from the standard curve showed that the sample had 8% the PDH activity of the control.



	Signal	% Control
Control	45871	100
Sample	2730	8

9. Specificity

Species Reactivity: Human, mouse, rat, porcine and bovine

10. Troubleshooting

Signal is saturated

It is very important that the amount of sample used is within the working range of the assay (use a best fit line for interpolation). For this reason, it is crucial to determine the working range for your sample type and avoid the region of signal saturation.

Signal is too weak

This occurs when the sample lacks measurable amounts of the protein. Increase the signal by adding more sample protein to another dipstick, or leave the dipstick in the activity solution for longer to maximize the signal

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