



**ab109883 –**

# **Pyruvate dehydrogenase (PDH) Protein Quantity Dipstick Array**

## **Instructions for Use**

For the measurement of Pyruvate dehydrogenase (PDH) protein in Human, Mouse, Rat and Bovine samples

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



# Table of Contents

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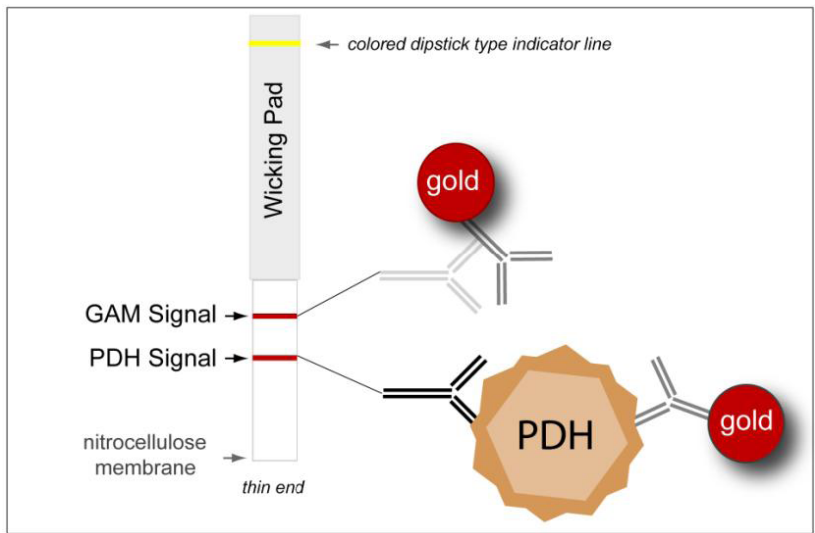
1. Introduction	3
2. Assay Summary	5
3. Kit Contents	6
4. Storage and Handling	6
5. Additional Materials Required	7
6. Preparation of Samples	7
7. Dipstick Procedure	9
8. Data Analysis	11
9. Specificity	13
10. Troubleshooting	14

# 1. Introduction

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ab109883 is used to measure the levels of assembled PDH (pyruvate dehydrogenase). PDH is present in all tissues; it plays a central role in metabolism as it is a key regulatory enzyme that functions at the junction between glycolysis and the tricarboxylic acid cycle. The PDH complex is composed of multiple copies of three catalytic component enzymes; pyruvate dehydrogenase or E1 (EC 1.2.4.1), dihydrolipoamide transacetylase or E2 (EC 2.3.1.12) and dihydrolipoamide dehydrogenase or E3 (EC 1.8.1.4). Inherent to its regulatory function, a number of other proteins regulate PDH activity. One of these, dihydrolipoamide dehydrogenase-binding protein (E3Bp) is necessary for the interaction of the E2 and E3 components.

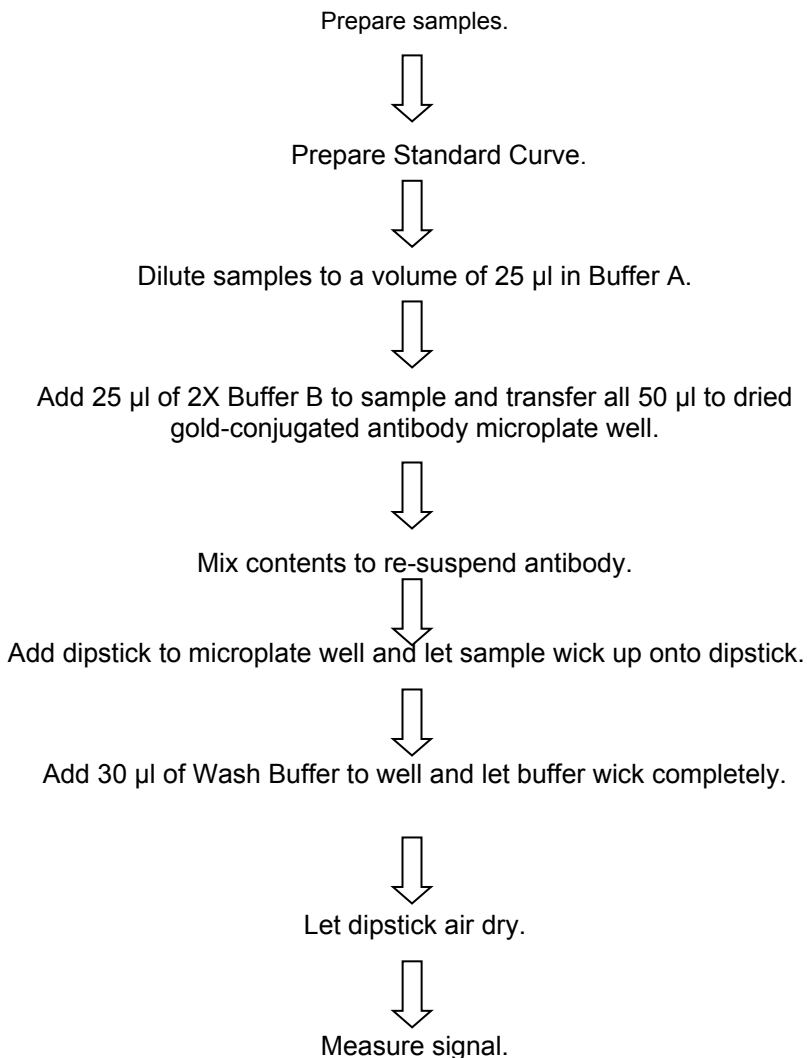
This dipstick assay kit utilizes two monoclonal antibodies. One recognizes the E2 enzyme (monoclonal antibody bound to the dipstick) and the other antibody (gold-conjugated) recognizes both the E2 and E3Bp components of the PDH complex. The signal intensity is measured by a dipstick reader or analyzed by other imaging systems such as a flatbed scanner. The kit is compatible with a variety of sample types (tissue or cell culture) from a number of different species (human, bovine, mouse, and rat).



**Figure 1.** Illustration of a PDH dipstick after signal development. The upper line on the dipstick is a positive control, which utilizes a goat anti-mouse (GAM) antibody that recognizes the gold-conjugated mouse monoclonal antibody. The PDH enzyme complex is captured by the mouse monoclonal antibody on the nitrocellulose membrane. A second, gold-conjugated mouse monoclonal antibody, specific to PDH, produces a signal at the antibody capture line. The signal intensity is proportionate to the amount of PDH present in the sample.

## 2. Assay Summary

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### 3. Kit Contents

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Sufficient materials for 90 or 30 measurements.

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Item	Quantity	
Dipsticks	90	30
Gold-conjugated antibody (dried in microplate wells)	90 wells	30 wells
Buffer A (Extraction buffer)	3 x 15 ml	1 x 15 ml
Buffer B (10X Blocking buffer)	3 x 0.4 ml	1 x 0.4 ml
Wash Buffer	3 x 2 ml	1 x 2 ml

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### 4. Storage and Handling

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All components are stable in their provided containers at room temperature out of direct sunlight. After diluting the 10X Blocking Buffer to 2X, store at 4°C. For long-term storage, all buffers can be stored at 4°C.

## 5. Additional Materials Required

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

## 6. Preparation of Samples

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*Note: Samples must be kept on ice. Proteases inhibitors are not provided.*

### A. Tissue Sample Preparation

1. Start with approximately 25 mg of tissue sample. Add 5-10 volumes of Buffer A per microgram of sample (e.g. if the sample weighs 100 mg, add 500-1,000  $\mu$ l of Buffer A).
2. Homogenize the sample.
3. Keep on ice for 20 minutes, mixing intermittently.
4. Spin the cell extract in a microcentrifuge at 13,000-16,000 rpm at 4°C for 20 minutes to pellet cellular debris.



5. Transfer supernatant to a new tube and determine protein concentration (e.g. BCA assays are recommended as the detergent in Buffer A does not affect the reading in the assay).
6. Proceed directly to Dipstick Procedure or freeze samples at  $-80^{\circ}\text{C}$ .

## **B. Cell Culture Sample Preparation**

1. Start with a cell pellet. Add 5-10 volumes of Buffer A to the cell pellet and mix (e.g. if the total sample volume displaces 50  $\mu\text{l}$  of volume, then add 250-500  $\mu\text{l}$  of Buffer A).
2. Keep on ice for 20 minutes, mixing intermittently.
3. Spin the cell extract in a microcentrifuge at 13,000 to 16,000 rpm for 20 minutes at  $4^{\circ}\text{C}$  to pellet cellular debris.
4. Transfer supernatant to a new tube and determine protein concentration (e.g. BCA assays are recommended as the detergent in Buffer A does not affect the reading in the assay).
5. Proceed directly to Dipstick Procedure or freeze samples at  $-80^{\circ}\text{C}$ .

**Note:** Samples prepared according to the protocol for ab109882 (Pyruvate dehydrogenase (PDH) Enzyme Activity Dipstick Assay Kit), may also be used in the Dipstick Procedure.

## 7. Dipstick Procedure

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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.

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<b>Sample Type</b>	<b>Suggested Working Range (<math>\mu\text{g}</math>)</b>
Human Fibroblasts	1.25 – 25 $\mu\text{g}$
Human HepG2	0.5 – 20 $\mu\text{g}$
Human skeletal muscle	0.5 – 15 $\mu\text{g}$
Mouse tissue	1 – 20 $\mu\text{g}$
Rat tissue	1 – 20 $\mu\text{g}$
Porcine tissue	0.5 – 10 $\mu\text{g}$

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**Table 1.** *Suggested working range for different sample types*

1. Add Buffer A to the sample to bring volume up to 25  $\mu$ L.
2. Make 2X Buffer B by adding 1.6 mL H<sub>2</sub>O to 0.4 mL 10X Buffer B.
3. Add the sample and 25  $\mu$ L of 2X Buffer B to a microplate well of dried gold-conjugated antibody, for a total sample volume of 50  $\mu$ L. Incubate the sample for 5 minutes, allowing the gold-conjugate to hydrate.
4. Re-suspend the dried gold-conjugated secondary antibody using a pipette or gently vortex.
5. Gently add a dipstick to the microplate well (place the thin/nitrocellulose end of dipstick down). The dipstick must reach the bottom of the well.
6. Allow the sample wick up onto the dipstick until the entire sample volume has been absorbed (the positive control band typically appears within 3 minutes. A 50  $\mu$ L sample should be wicked up completely in 20 minutes; more viscous extracts may take longer. The dipstick must absorb the entire sample volume before Wash Buffer is added.

7. Add 30  $\mu$ l of Wash Buffer and let the entire buffer volume wick up onto the dipstick.
8. Leave dipstick to air-dry.
9. Measure the signal intensity with a dipstick reader or other imaging system, e.g. flat-bed scanner.

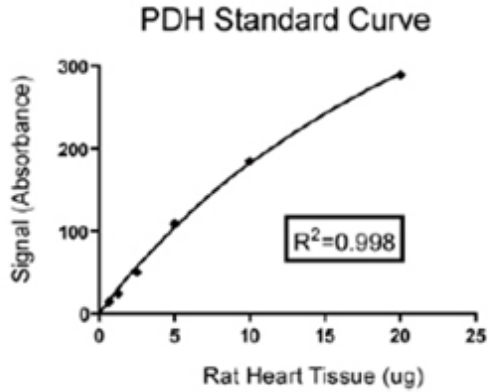
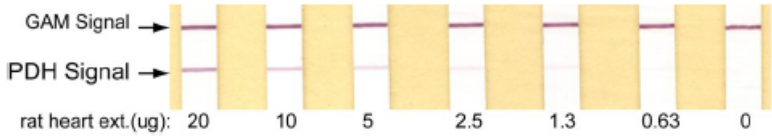
## 8. Data Analysis

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Illustrated is an experiment using the ab109883 assay kit to quantify PDH levels using various concentration of rat tissue protein extract. Protein extract was either: undiluted (control), diluted 1:2 (Sample A), or diluted 1:10 (Sample B). Samples were prepared as described in this protocol. All data were analyzed using a Dipstick Reader and GraphPad software.

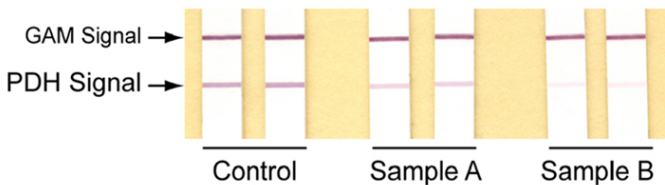
### A. Generating a standard curve

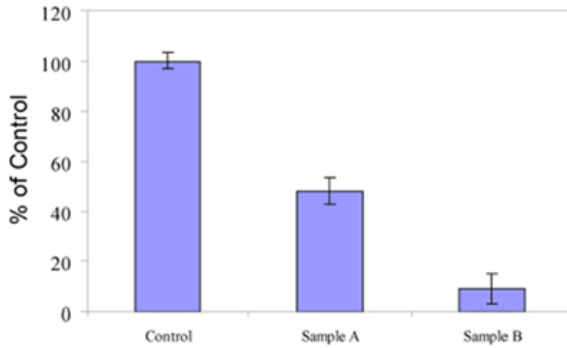
Shown is a 1:2 dilution series using a positive control sample (see Table 1 for recommended protein ranges). Approximately 7 to 8 dipsticks are suitable for covering the entire working range and the blank for background levels. In this example the dilution series starts with 20  $\mu$ g of rat heart tissue extract. A one-site hyperbola line was generated for best-fit analysis using GraphPad.



**B. Analysis of samples.**

Based on the above standard curve values, 18  $\mu$ g (control), 9  $\mu$ g (sample A) and 1.8  $\mu$ g (sample B) of extract were loaded to each dipstick. Two dipsticks per sample were run (typical intra-assay precision is 90-98%).





Using GraphPad software, the signal intensity from the standard curve was interpolated and the quantity of PDH in samples A and B was determined. Based on the above analysis, the unknown samples, A and B, had 48% and 9% of control PDH levels, respectively.

## 9. Specificity

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Species Reactivity: Human, mouse, rat, and bovine

## 10. Troubleshooting

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### **Signal is saturated**

It is very important that the amount of sample used is within the working range of the assay (a best fit line for interpolation as generated with the GraphPad program). Therefore, it is crucial to know the working range for the sample type and avoid the region of signal saturation (see Table 1). Determination of the working range is recommended for the sample in case of signal saturation.

### **Signal is too weak**

This occurs when the sample lacks measurable amounts of the protein. To increase signal add more sample to another dipstick.

### **Positive control band does not appear**

Make sure that the wicking paper is in contact with the nitrocellulose membrane. Care should always be taken when handling the dipsticks to prevent disruption of this junction (see Figure 1).

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