

Pyruvate Dehydrogenase (PDH) Protein Quantity Dipstick Assay Kit

MSP31

Rev.1

DESCRIPTION

Pyruvate Dehydrogenase (PDH) Protein Quantity Dipstick Assay Kit

Sufficient materials are provided for 30 (MSP31-30) or 90 (MSP31-90) measurements.

Kit Contents:

Item	MSP31-30	MSP31-90
Dipsticks	30	90
Gold-conjugated antibody (dried in microplate wells)	30 wells	90 wells
Buffer A* (Extraction buffer)	15 mL	45 mL
Buffer B* (Blocking buffer)	1 mL	3 mL
Buffer C* (Wash buffer)	1 mL	3 mL

Storage:

Store dipsticks and microplate with gold-conjugated antibody at room temperature out of direct sunlight in their provided containers. High humidity conditions should be avoided. Store Buffers A, B, and C at 4°C, or at -20°C for long term storage. *Buffers A, B, and C are interchangeable with Buffers A, B, and C from other Quantity Dipstick Assay Kits (MS131, MS133, MS431, MSF31).

INTRODUCTION

The Pyruvate Dehydrogenase (PDH) Protein Quantity Dipstick Assay Kit (MSP31) 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 (MitoSciences' MS1000 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).

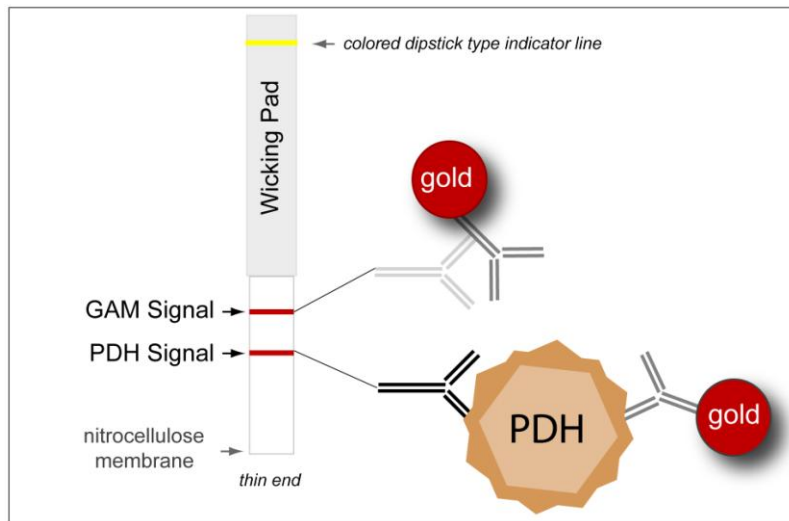


Figure 1. Illustration of a PDH dipstick after signal development. The upper line on the dipstick is a positive control, which utilizes a goat anti-mouse (GAM) antibody that recognizes the gold-conjugated mouse monoclonal antibody. The PDH enzyme complex is captured by the mouse monoclonal antibody on 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.

ADDITIONAL MATERIALS REQUIRED

- Dipstick reader (see MitoSciences' MS1000 Dipstick Reader) or other imaging system
- Method for determining protein concentration
- Pipetting devices
- Protease inhibitors

DIPSTICK ASSAY PROTOCOL

A. Sample Preparation

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

1. Tissue Sample Preparation

- a. 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 μ L of Buffer A).
- b. Homogenize the sample.
- c. Keep on ice for 20 minutes, mixing intermittently.
- d. Spin the cell extract in a microcentrifuge at 13,000-16,000 rpm at 4°C for 20 minutes to pellet cellular debris.
- e. 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).
- f. Proceed directly to Part B of the Protocol or freeze samples at -80°C.

2. Cell Culture Sample Preparation

- a. 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 μ L of volume, then add 250-500 μ L of Buffer A).
- b. Keep on ice for 20 minutes, mixing intermittently.
- c. Spin the cell extract in a microcentrifuge at 13,000-16,000 rpm at 4°C for 20 minutes to pellet cellular debris.
- d. 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).
- e. Proceed directly to Part B of the protocol or freeze samples at -80°C.

Note: *Samples prepared according to the protocol for MSP30 (Dipstick Assay Kit for PDH Activity), may also be used in Part B.*

B. 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.

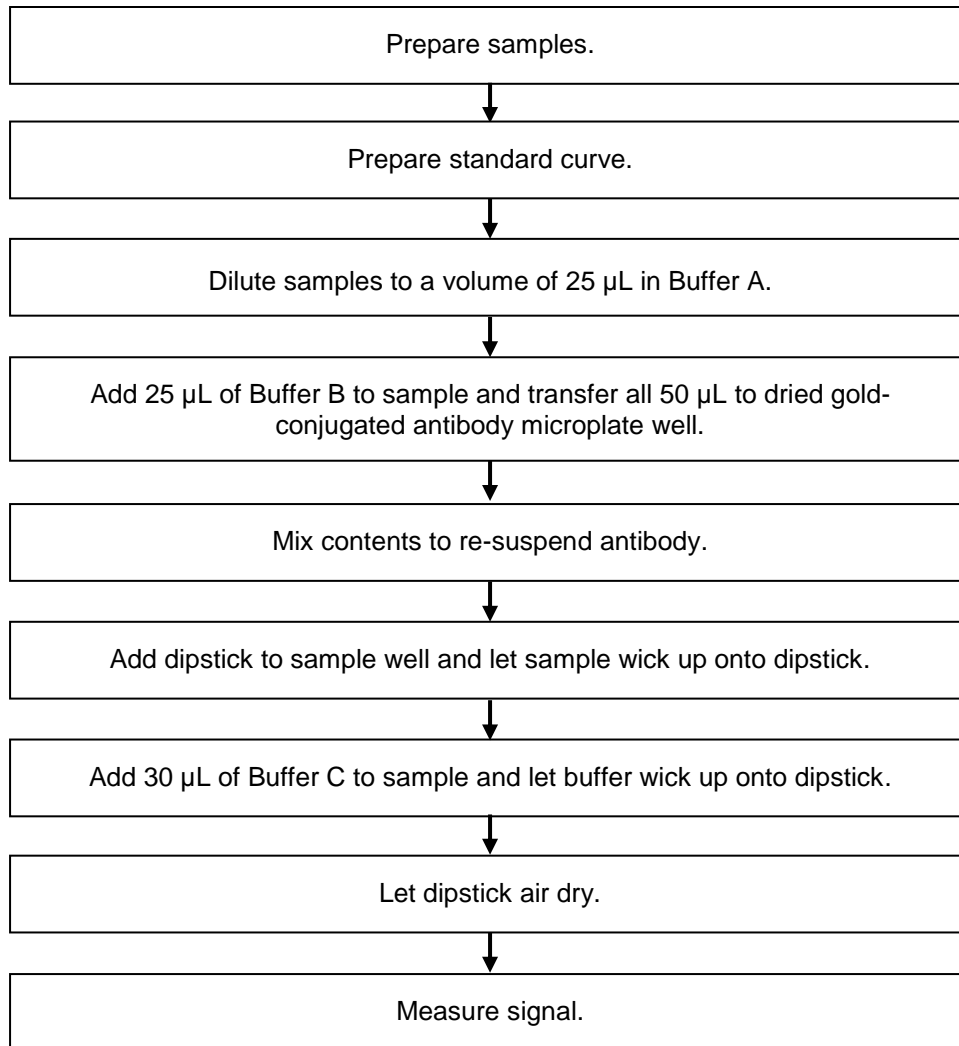
Sample Type	Working Range
Human fibroblasts	1.25 – 25 µg
Human HepG2	0.5 – 20 µg
Human skeletal muscle	0.5 – 15 µg
Mouse tissue	1 – 20 µg
Rat tissue	1 – 20 µg
Porcine tissue	0.5 – 10 µg

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

1. Add Buffer A to the sample to bring volume up to 25 µL.
2. Add the sample and 25 µL of Buffer B to a microplate well of dried gold-conjugated antibody, for a total sample volume of 50 µL. Incubate the sample for 5 minutes, allowing the gold-conjugate to hydrate.
3. Re-suspend the dried gold-conjugated secondary antibody using a pipette.
4. Gently add a dipstick to the microplate well, placing the thin/nitrocellulose end of the dipstick down. The dipstick has to reach the bottom of the well.
5. Allow the sample to wick up onto the dipstick until the entire sample volume has been absorbed. The positive control band typically appears within 3 minutes. A 50 µL sample should be wicked up completely in 20 minutes; more viscous extracts may take longer. **The dipstick must absorb the entire sample volume before Buffer C is added.**
6. Add 30 µL of Buffer C and let the entire buffer volume wick up onto the dipstick.
7. Leave dipstick to air-dry.
8. Measure the signal intensity with a dipstick reader (recommended: MitoSciences MS1000 Dipstick Reader) or other imaging system, e.g. flat-bed scanner.

FLOW CHART

For quick reference only. Be completely familiar with the previous details of this protocol before performing the assay.

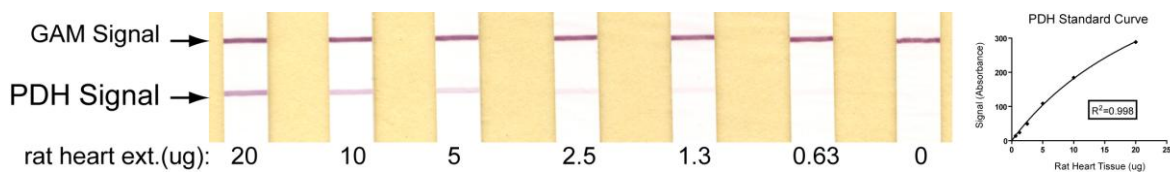


EXAMPLE EXPERIMENT

Illustrated is an experiment using the MSP31 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 MitoSciences MS1000 Dipstick Reader and GraphPad software.

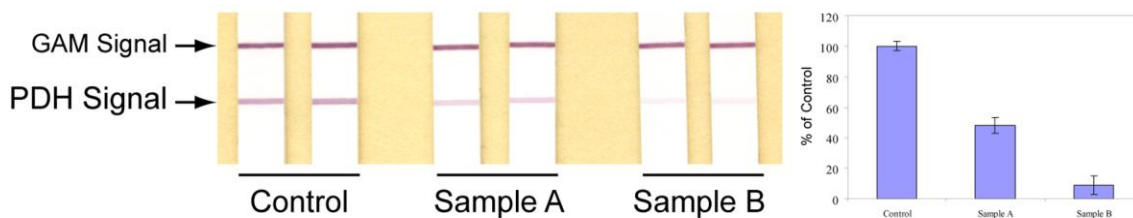
Step 1. Generating a standard curve

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 μg of rat heart tissue extract. A one-site hyperbola line was generated for best-fit analysis using GraphPad.



Step 2. Analysis of samples

Based on the above standard curve values, 18 μg (control), 9 μg (sample A) and 1.8 μg (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.

TROUBLESHOOTING GUIDE

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).