

**ab110671 –**

**Pyruvate dehydrogenase  
(PDH) Combo  
(Activity + Profiling)  
Microplate Assay Kit**

Instructions for Use

For the activity and relative quantity of PDH in a Human, Bovine, Mouse or Rat sample

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



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# 1. Introduction

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ab110671 (MSP20) combines 2 independent kits for PDH activity and PDH relative protein quantity: ab109902/MSP18 (PDH Enzyme Activity Microplate Assay Kit) and ab110174/MSP19 (PDH Protein Profiling Microplate Assay Kit). ab110671 contains the complete contents of each of these two kits, which can be run in parallel to determine not only the relative PDH protein concentration and PDH activity in unknown samples, but also the relative specific activity (Activity/Protein Quantity).

Included in these kits are the necessary buffers and detergent to solubilize PDH from mitochondria, whole tissue homogenates or cultured cells, two microplates (one each for ab109902 and ab110174) with wells pre-coated with a PDH-binding antibody, antibodies and label to detect PDH protein immunocaptured in the microwells of one plate (ab110174), and reagent mix for the PDH enzyme reaction in the second plate (ab109902). Each ab109902 PDH Enzyme Activity Kit and each ab110174 PDH Protein Profiling Kit contains enough material to perform 96 tests. Since the microplates are arranged as 12 strips of 8 wells, up to 12 separate experiments can be performed with each plate.

## 2. Assay Summary

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### ENZYME ACTIVITY ASSAY SUMMARY

#### Prepare Reagents (approximately 1 hour)

- Adjust sample concentration to the appropriate concentration in PBS, i.e., 5.3 mg/ml for mitochondria, 23.7 mg/ml for whole tissues, and 15 mg/ml for cultured cells, in PBS.
- Perform Detergent extraction with the appropriate amount of Detergent.



#### Load Plate (3 hours)

- Load sample(s) on plate being sure to include a normal sample and a buffer control as null reference.
- Incubate 3 hrs at room temperature.



#### Measure (30 minutes)

- Rinse wells twice with 1x Buffer.
- Make sufficient Assay Solution to load 200  $\mu$ l/well.
- Add 200  $\mu$ l of Assay Solution to each well.
- Measure OD450 at 20 seconds intervals for 30 minutes.

## **PROTEIN PROFILING ASSAY SUMMARY**

### **Prepare Sample (approximately 1 hour)**

- Adjust sample concentration to the appropriate concentration in PBS, i.e., 5.3 mg/ml for mitochondria, 23.7 mg/ml for whole tissues, and 15 mg/ml for cultured cells, in PBS.
- Perform Detergent extraction with the appropriate amount of Detergent.



### **Load Plate (3 hours)**

- Load sample(s) on plate being sure to include a normal sample and a buffer control as null reference.
- Incubate 3 hrs at room temperature.



### **Antibody binding and measurement (2.5 hours)**

- Wash wells twice with 1X Stabilizer.
- Add 1X Detector antibody to each well. Incubate 1 hour.
- Wash wells twice with 1X Buffer.
- Add 1X HRP Label to each well. Incubate 1 hour.
- Wash wells three times with 1X Buffer.
- Add Development Solution to each well.
- Measure OD<sub>600</sub> (kinetic) approximately every minute for 30 min at room temperature or OD<sub>450</sub> (endpoint after stopping with 1 N HCl).

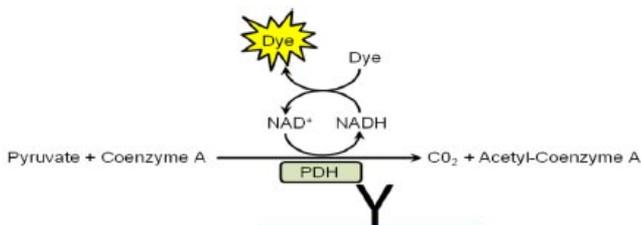
### 3. Enzyme Activity Assay

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Pyruvate Dehydrogenase (PDH) Enzyme Activity Microplate Assay Kit (ab109902/MSP18)

#### INTRODUCTION:

ab109902 Pyruvate Dehydrogenase (PDH) Enzyme Activity Microplate Assay Kit (MSP18) is used to determine the activity of pyruvate dehydrogenase (PDH) in a sample. The PDH enzyme is immunocaptured within the wells of the microplate and PDH activity is determined by following the reduction of  $\text{NAD}^+$  to  $\text{NADH}$ , coupled to the reduction of a reporter dye to yield a colored (yellow) reaction product whose concentration can be monitored by measuring the increase in absorbance at 450 nm (Figure 1).



**Figure 1:** PDH activity assay reaction scheme

PDH is the key regulatory enzyme of cellular metabolism because it links the TCA cycle and subsequent oxidative phosphorylation with glycolysis and gluconeogenesis as well as with both lipid and amino acid metabolism. PDH activity is regulated by PDK-dependent phosphorylation and PDP-dependent dephosphorylation of PDH. Phosphorylation inactivates PDH whereas dephosphorylation activates PDH.

ab109902 can be used to elucidate various aspects of PDH activity, physiologic regulation and phosphorylation status. Three applications are described briefly below:

- 1. Specialized sample preparation.** PDH activity in cells and tissues is regulated by reversible phosphorylation (PDH inhibition) and dephosphorylation (activation) caused by endogenous PDH kinases and PDH phosphatases. ab109902 does not include PDH kinase or PDH phosphatase inhibitors. However, these reagents may be incorporated into the sample preparation and/or sample dilution buffers at the researcher's discretion to preserve the ENDOGENOUS phosphorylation and activity status of PDH during cell and tissue solubilization.
- 2. Reagents to manipulate the phosphorylation status and activity of PDH AFTER immunocapture.** Exogenous PDH Kinases and PDH phosphatases can be used to manipulate

the phosphorylation status and activity of immunocaptured PDH. For example, post-capture treatment of immunocaptured PDH with appropriate amounts of PDP1 or PDP2 will fully dephosphorylate the enzyme, resulting in maximum enzyme activity and thus allow measurement of TOTAL PDH activity in a sample. A comparison of TOTAL and ENDOGENOUS PDH activity in a sample allows one to calculate the amount (%) of PDH activity that is inhibited by phosphorylation at the time of immunocapture. Note that PDH activity can also be inhibited by other factors, such as oxidative stress, that are not affected by phosphorylation state. Abcam offers a complete sections of recombinant human PDKs and PDPs that can be used in conjunction with ab109902:

- ab110359 (MSP41): PDH Kinase-1 (PDK1), recombinant human
- ab110354 (MSP42) : PDH Kinase-1 (PDK2), recombinant human
- ab110355 (MSP43): PDH Kinase-1 (PDK3), recombinant human
- ab110356 (MSP44): PDH Kinase-1 (PDK4), recombinant human
- ab110357 (MSP45): PDH Phosphatase-1 (PDP1), recombinant human
- ab110358 (MSP46): PDH Phosphatase-2 (PDP2), recombinant human

### **3. High-Throughput Screening of PDK Kinase inhibitors.**

The PDKs regulate PDH activity under normal physiologic conditions, such as in response to exercise and diet, but also play a major role in dysregulation of metabolism in disease such as diabetes and cancer. ab109902 can be used in conjunction with each of the specific PDKs to aid identification of drugs that regulate PDH kinase activities, namely in development of specific inhibitors of the different PDH kinases as potential treatments for cancer, diabetes, or to identify off-target inhibition of PDKs by kinases directed at other targets.

#### **KIT CONTENTS:**

Included in this kit is the necessary buffer and detergent for sample preparation, and reagents for the enzyme reaction. The kit contains a 96-well microplate with an anti-PDH monoclonal antibody pre-bound to the wells of the microplate. This plate can be broken into 12 separate 8-well strips for convenience; therefore the plate can be used for up to 12 separate experiments.

<b>Item</b>	<b>Quantity</b>	<b>Storage</b>
20X Buffer	15 ml	4°C
Detergent	2 x 1 ml	4°C
20X Reagent Mix	2 x 0.6 ml	-80°C
5X Stabilizer	13 ml	-20°C
100X Coupler	0.25 ml	-80°C
100X Reagent Dye	0.25 ml	-20°C
96-well microplate (12 strips)	1	4°C

**STORAGE AND HANDLING:**

See Table of Kit Contents.

NOTE: Avoid freeze-thaw cycles of frozen components by making appropriate aliquots if the entire plate is not run in a single 96-well experiment.

**ADDITIONAL MATERIALS REQUIRED:**

- Spectrophotometer measuring absorbance at 450 nm
- Deionized Water
- Multichannel Pipetting devices
- Protein assay method
- Phosphate buffered saline solution (PBS)

1.4 mM  $\text{KH}_2\text{PO}_4$   
8 mM  $\text{Na}_2\text{HPO}_4$   
140 mM NaCl  
2.7 mM KCl, pH 7.3

## **PREPARATION OF SAMPLES:**

ab109902 PDH Enzyme Activity Microplate Assay Kit has been developed for use with human samples. Bovine, mouse, or rat materials are also compatible. Other species have not been tested. Suitable samples include mitochondria, whole tissue extracts and whole cultured cell extracts.

The protein concentration of the sample must be measured prior to sample solubilization. Once diluted to the specified concentration the sample is detergent solubilized and diluted to within the linear range of measurement. A control or normal sample should always be included in the assay as a reference measurement. In addition, a buffer control should be used as a negative control.

1. Mitochondria and whole tissues should be homogenized in PBS, while cultured cell pellets should be suspended in PBS. The protein concentration should then be determined using a standard method such as the BCA method. Then, use PBS to adjust the sample concentrations as follows:

5.3 mg/ml for mitochondria

23.7 mg/ml for tissue homogenates

15 mg/ml for cultured cells

*(Approximate numbers of cells/mg protein are given in the frequently asked questions section on page 18).*

2. Solubilize intact, functional PDH by adding Detergent to the sample as described below.

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<b>Components</b>	<b>Purified mitochondria at 5.3 mg/ml</b>	<b>Tissue homogenates at 23.7 mg/ml</b>	<b>Cultured cells at 15 mg/ml</b>
Sample	19 volumes	19 volumes	9 volumes
Detergent	1 volume	1 volume	1 volume
Final Protein Concentration (mg/ml)	5	22.5	13.5

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3. Incubate on ice for 10 minutes.
4. Centrifuge in a tabletop centrifuge for 10 minutes at 4°C as specified below. Carefully collect and save the supernatant. Discard the pellet.

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<b>Sample type</b>	<b>RCF (x g)</b>
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Purified mitochondria	5000
Tissue homogenates	1000
Cultured cells	1000

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5. Add 10 ml of 20X Buffer to 190 ml deionized H<sub>2</sub>O. Label this mixture as 1X Buffer.
6. Dilute all samples to the desired concentration in 1X Buffer. The table below shows the linear working range for the assay using various samples. The working range for your sample set should be confirmed by testing a representative reference control sample at a series of dilutions across the expected working range. Results from individual experimental samples can then be compared directly when tested at concentrations within the working range.

### **Recommended Sample Dilutions**

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Mitochondria extracts	10-100 $\mu$ g / 200 $\mu$ l/well
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Whole tissue extracts	20-100 $\mu$ g / 200 $\mu$ l/well
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Cultured cells extracts	100-1000 $\mu$ g / 200 $\mu$ l/well
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## **ASSAY METHOD:**

### **A. Plate Loading:**

1. Add 200  $\mu$ l of sample prepared in Preparation of Samples Step 6 to each well of the microplate that will be used for this experiment. Be sure to include a normal or control sample in addition to a buffer control as a negative control.
2. Incubate microplate for 3 hours at room temperature.

### **B. Measurement:**

1. Prepare 1X Stabilizer by mixing 1 volume of thawed 5X Stabilizer with 4 volumes of 1X Buffer.
2. Prepare "Assay Solution" as described in the table below. Warm to room temperature. NOTE: Thaw 20X Reagent Mix immediately before use. If the 20X Reagent Mix is to be used in multiple experiments with fewer than 12

strips/experiment, aliquot and store unused 20X Reagent Mix at -80°C immediately after thawing.

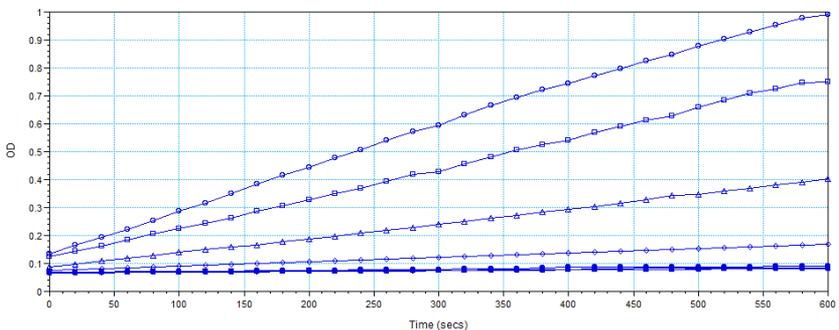
<b>No. of Strips</b>	<b>20X Reagent Mix (µl)</b>	<b>1X Buffer (ml)</b>	<b>100X Coupler (µl)</b>	<b>100X Reagent Dye (µl)</b>
1	88	1.63	18	18
2	175	3.25	35	35
3	263	4.89	53	53
4	350	6.51	70	70
5	438	8.14	87	87
6	525	9.77	105	105
7	612	11.39	123	123
8	700	13.02	140	140
9	788	14.65	158	158
10	875	16.28	175	175
11	963	17.90	192	192
12	1050	19.53	210	210

3. Empty the wells of the microplate and to each well add 300 µl of 1X Stabilizer.

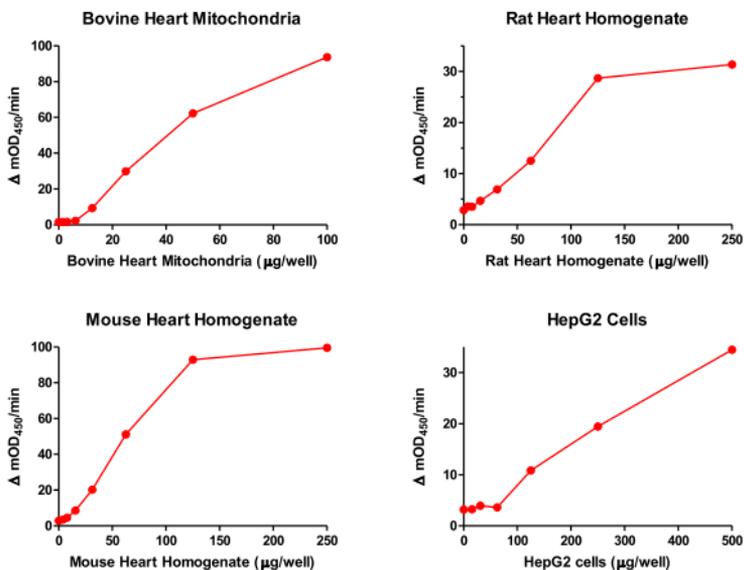
4. Again, empty the wells of the microplate and to each well add 300  $\mu$ l of 1X Stabilizer.
5. Empty the wells again.
6. Add 200  $\mu$ l of Assay Solution to each well carefully to avoid bubbles. Any bubbles should be popped with a fine needle as rapidly as possible.
7. Measure the absorbance of each well at 450 nm at room temperature using a kinetic program for approximately 15 minutes. The interval between readings should be as short as your reader allows but not longer than 1 minute between reads. Incorporate a shake step between reads if possible.

## DATA ANALYSIS:

PDH activity is expressed as the initial rate of reaction, determined from the slopes of the curves generated.



**Figure 2:** Example of microplate reader recorded data. Bovine heart mitochondria were loaded at 100  $\mu\text{g}/\text{well}$  (top trace) and 2-fold dilutions (stepwise lower traces). Activity should always be related to a control or normal sample to obtain the relative activity of PDH in experimental samples.



**Figure 3:** Mitochondria, tissue extracts and whole cultured cell extracts all show linear relationships between signal and sample load at limiting concentrations. The rates shown were determined as change in OD over time, and these are best represented as change in milliOD per minute.

**SPECIFICITY:**

Species Reactivity: Human, Rat, Mouse, Bovine

**REPRODUCIBILITY:**

Typical intra-assay variations (same day, same sample) <10%.

## FREQUENTLY ASKED QUESTIONS:

Question	Answer
How should I store my Reagent Mix?	Reagent mix is temperature sensitive. It must be stored at $-80^{\circ}\text{C}$ and is stable for at least six months from date of receipt. If the reagent mix is to be used in multiple experiments with fewer than 12 strips/experiment, aliquot and store unused reagent mix at $-80^{\circ}\text{C}$ immediately after thawing.
How should I aliquot my Reagent Mix?	Divide the reagent mix into equal aliquots depending on how many experiments you wish to run. The plate comes in 12 strips, therefore it is anticipated that up to 12 experiments on different days could be done. The reagent mix is supplied as $2 \times 0.5$ ml aliquots, thus for 12 independent experiments each tube of the reagent mix can further be divided into $6 \times 83$ $\mu\text{l}$ aliquots and immediately frozen at $-80^{\circ}\text{C}$ for storage until use.

<p>How do I optimize sample solubilization?</p>	<p>Mitochondria have two membrane bilayers which must be dissolved by detergent before PDH can be isolated. PDH is a huge, extremely complex, multi-subunit enzyme which is sensitive to high detergent concentrations. Therefore, the correct detergent: protein ratio must be used to efficiently solubilize the mitochondrial outer and inner membranes while maintaining PDH integrity. This kit suggests optimized ratio for specific sample types. However, this ratio may need to be adjusted from sample to sample, particularly when contaminated with excess extracellular proteins such as serum. Therefore, it may be necessary to adjust the concentration of detergent or isolated mitochondria.</p>
<p>How do I isolate mitochondria?</p>	<p>We have found that little or no optimization is necessary if crude mitochondria are made from samples. Mitochondria can be prepared by simple differential centrifugation of homogenized samples.</p>
<p>How do I grow and prepare cultured cell samples?</p>	<p>PDH activity in cells from different origins differs greatly. Cells grown in glucose have a lower activity than those grown in galactose/glutamine. Consequently, cell type and growth conditions are a large factor in PDH activity measured.</p>

<p>Approximately how much protein is yielded from my plate of cells?</p>	<p>We find the following typical yield of cells from a single confluent 177 cm<sup>2</sup> plate:  Human fibroblasts: 1x10<sup>7</sup> cells, 1.5 mg total protein  Human HepG2: 2x10<sup>7</sup> cells, 3 mg total protein  It is recommended that you accurately determine from your first confluent plate the number of cells and the total protein yield.</p>
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## 4. Protein Profiling Assay

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Pyruvate Dehydrogenase (PDH) Protein Profiling Microplate Assay Kit (ab110174/MSP19)

### INTRODUCTION:

ab110174 (MSP19) Kit can be used to determine the relative amount of PDH protein in a sample. This assay is a ‘sandwich’ ELISA, where the PDH enzyme is purified and immobilized by an anti-PDH capture antibody pre-coated in the microplate wells. The amount of captured PDH is determined by adding a second (detector) anti-PDH antibody which binds to the captured PDH, followed by binding of an HRP conjugated goat anti-mouse antibody that binds the detector anti-PDH antibody. The detector-bound HRP then changes the colourless HRP development solution to blue and the colour intensity (absorbance) is proportional to the amount of PDH captured.

ab110174 PDH Profiling Quantity Assay has been developed for use with human samples but bovine, mouse, and rat materials are also compatible. Other species have not been tested. Importantly, it is suitable for use with whole tissue or cell lysates without the need for mitochondrial isolation.

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Tissue extracts	0.5 - 25 $\mu$ g / 200 $\mu$ l
Cultured cell extracts†	0.5 - 50 $\mu$ g / 200 $\mu$ l

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**Table 1.** Typical ranges of measurement.

† Mitochondrial PDH quantity is controlled by cellular metabolism. Consequently, cells with different metabolic requirements, such as those derived from different tissues, vary widely in their PDH amount. Additionally, cells of the same kind but cultured in different growth conditions show similar effects. For example, cells grown in glucose-rich media derive most of their energy by glycolysis. Cells grown in carbon sources which promote oxidative phosphorylation (such as galactose/glutamine), upregulate mitochondrial enzymes, including PDH. Ultimately, the cell type and growth conditions must be chosen carefully to obtain PDH quantity measurements.

PDH is the key regulatory enzyme of cellular metabolism because it links the TCA cycle and subsequent oxidative phosphorylation with glycolysis and gluconeogenesis as well as with both lipid and amino acid metabolism. PDH activity is regulated primarily by PDK-dependent phosphorylation and PDP-dependent dephosphorylation

of PDH. Phosphorylation inactivates PDH whereas dephosphorylation activates PDH. Phosphorylation occurs at Serines 232, 293, and 300 of the human E1 $\alpha$  subunits.

Abcam also offers a comprehensive line of PDH-related assays and reagents that can be used in conjunction with ab110174 (MSP19) to elucidate various aspects of PDH activity, physiologic regulation and phosphorylation status. These include all four PDH kinases, both PDH phosphatases, PDH activity microplate assays and PDH protein quantity microplate assays. For convenience, these tools are available combined in several kits and described in additional protocols.

**KIT CONTENTS:**

Detergent and buffer to solubilize PDH from mitochondria, whole tissue homogenates or cultured cells, a microplate with wells pre-coated with a PDH binding antibody, antibodies and enzyme label to detect PDH immunocaptured in the microwells, and a reaction substrate/buffer to quantitate levels of bound antibody (PDH). The kit contains enough material to perform 96 tests. Since the plate is arranged as 12 strips of 8 wells, up to 12 separate experiments can be performed.

<b>Item</b>	<b>Quantity</b>	<b>Storage</b>
20X Buffer	20 ml	4°C
Detergent	1 ml	4°C
10X Blocking Buffer	10 ml	4°C
5X Stabilizer	13 ml	-20°C
20X Detector Antibody	1 ml	4°C
20X HRP Label	1 ml	4°C
1X HRP Development Solution	20 ml	4°C
96-well microplate (12 strips)	1	4°C

## **STORAGE AND HANDLING:**

5X Stabilizer should be stored at -20°C. Remainder of kit should be stored at 4°C. When stored the kit is stable for 6 months

## **ADDITIONAL MATERIALS REQUIRED:**

- Spectrophotometer that measures absorbance at 600nm
- Multichannel pipette (50 - 300 µl) and tips
- Protein assay method
- Phosphate buffered saline (PBS)
- Optional for 450 nm endpoint data measurement – 1 N HCl

## **PREPARATION OF SAMPLES:**

The protein concentration of the sample should be measured before solubilization. Once diluted to the specified concentration the sample is detergent-solubilized and diluted to within the linear range of measurement. A control or normal sample should always be included in the assay as a reference positive control measurement. In addition, a buffer control should be used as a negative control.

NOTE: If phospho-serine detector antibodies are used in place of the standard PDH detector mAb, it is critical to inhibit the endogenous PDH phosphatases and kinases during sample preparation and immunocapture to ensure the phosphorylation status of the sample does not change during processing.

1. Mitochondria and whole tissues should be homogenized in PBS, while cultured cell pellets should be suspended in PBS. The protein concentration should then be determined using a standard method such as BCA method. Then, use PBS to adjust the sample concentrations as follows:

5.3 mg/ml for mitochondria

23.7 mg/ml for tissue homogenates

15 mg/ml for cultured cells

*(Approximate numbers of cells/mg protein are given in the frequently asked questions section on page 31).*

2. Solubilize intact, functional PDH by adding Detergent to the samples as described below.

<b>Component</b>	<b>Purified mitochondria at 5.3 mg/ml</b>	<b>Tissue homogenates at 23.7 mg/ml</b>	<b>Cultured cells at 15 mg/ml</b>
Sample	19 volumes	19 volumes	9 volumes
Detergent	1 volume	1 volume	1 volume
Final Protein Concentration (mg/ml)	5.0	22.5	13.5

3. Incubate on ice for 10 minutes.

4. Centrifuge in a tabletop centrifuge for 10 minutes at 4°C as specified below. Carefully collect and save the supernatant. Discard the pellet.

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<b>Sample Type</b>	<b>RFC (x g)</b>
Purified mitochondria	5,000
Tissue homogenates	1,000
Cultured cells	1,000

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5. Add 15 ml of 20X Buffer to 285 ml deionized H<sub>2</sub>O. Label this mixture as 1X Buffer.
6. Prepare “Incubation Solution” by mixing 1 part 10X Blocking Buffer with 9 parts 1X Buffer (the total volume of Incubation Solution needed per experiment depends on the number of wells to be used in the experiment at hand).
7. Dilute all samples to the desired concentration in Incubation Solution. Table 1 (page 3) shows the working range for the assay using various samples. The working range for your sample set should be confirmed by testing a representative reference control sample at a series of dilutions across the expected working range. Results from individual experimental samples can then be compared directly when tested at concentrations within the working range.

## **ASSAY METHOD:**

### **Plate Loading and Assay Steps**

1. Load wells at 200  $\mu$ l per well with samples prepared in Section 6. Include a control (normal) sample as a positive control. Also include a buffer control (200  $\mu$ l Incubation Solution without sample) as a null or background reference.
  
2. Cover/seal the plate and incubate for 3 hours at room temperature.
  - a. During this time prepare the 1X Detector Antibody by mixing 1 part 20X Detector Antibody with 19 parts Incubation Solution.
  
  - b. Also prepare 1X Stabilizer by mixing 1 part 5X Stabilizer with 4 parts 1X Buffer.
  
3. Wash the plate

- a. Empty the wells by turning the plate over a receptacle and firmly shaking out the well contents in one rapid downward motion.
  - b. Rapidly add 300  $\mu$ l 1X Stabilizer to each well. The wells must not become dry during any step. Repeat this wash once more for a total of two washes in 1X Stabilizer. After the last wash strike the microplate surface onto paper towels to remove excess liquid.
4. Add 200  $\mu$ l of 1X Detector Antibody to each well used. Cover/seal the plate and incubate for 1 hour at room temperature.
  - a. During this time prepare 1X HRP label by mixing 1 part 20X HRP Label with 19 parts Incubation Solution.
5. Repeat the wash procedure in step 3 except this time use 1X Buffer (without Stabilizer) and do a **total of two washes in 1X Buffer**.
6. Add 200  $\mu$ l of 1X HRP Label to each well used. Cover/seal the plate and incubate for 1 hour at room temperature.

Meanwhile prepare the microplate spectrophotometer using the parameters described below.

- a. During this time, allow the HRP Development solution to warm to room temperature.
7. Repeat the wash procedure in step 5, but perform **a total of three washes with 1X Buffer.**
8. Rapidly add 200  $\mu$ l HRP Development solution to each empty well and record (at room temperature) blue color development in the prepared microplate reader immediately

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Mode:	Kinetic
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Wavelength:	600 nm
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Time:	Up to 15 min
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Interval:	20 sec to 1 min
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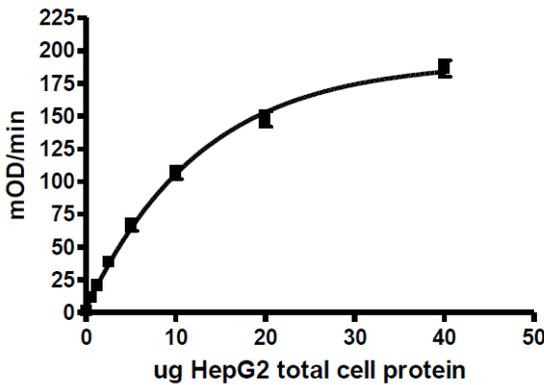
Shaking:	Shake between readings
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*Alternative – At a user defined colour development time, record the endpoint OD data at (i) 600 nm or (ii) stop the reaction by adding 50  $\mu$ l stop solution (1 N HCl) to each well and record OD at 450 nm.*

## **DATA ANALYSIS:**

Examine the color development over time in each well. Under the conditions stated above the color development should be linear over the 30 minute time period of measurement. Subtract the initial absorbance reading from the final absorbance reading to determine the quantity of PDH in each well. This quantity should always be related to a control or normal sample to obtain the relative quantity of PDH in experimental samples.



**Figure 1** is an example of the quantity of PDH capture from a HepG2 cultured cell lysate. The sample was diluted to show that over this range of concentrations that can be used. Each sample was measured in 6 replicates. Bars show standard deviations.

**SPECIFICITY:**

Species Reactivity: Human, Rat, Mouse, Bovine.

**REPRODUCIBILITY:**

Typical intra-assay variations (same day, same sample) <15%.

### FREQUENTLY ASKED QUESTIONS:

Question	Answer
How do I grow and prepare cultured cell samples?	The amount of PDH in cells from different origins differs greatly. Cells grown in glucose have a lower activity than those grown in galactose/glutamine. Consequently, cell type and growth conditions are a large factor in PDH activity measured.
Approximately how much protein is yielded from my plate of cells?	We find the following typical yield of cells from a single confluent 177 cm <sup>2</sup> plate: Human fibroblasts: 1 x 10 <sup>7</sup> cells, 1.5 mg total protein Human HepG2 :2 x 10 <sup>7</sup> cells, 3 mg total protein  It is recommended that you accurately determine from your first confluent plate the number of cells and the total protein yield







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