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# Product Information

**Pyruvate Dehydrogenase Activity Assay Kit**

Catalog Number **MAK183**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Pyruvate dehydrogenase (PDH) is a mitochondrial enzyme that catalyzes the conversion of pyruvate to acetyl-CoA and $CO_2$, and also links the tricarboxylic acid (TCA) and glycolysis pathways. The enzyme is inhibited by phosphorylation and activated by dephosphorylation.[1] Mutations in PDH have been linked to pyruvate dehydrogenase deficiency (causing lactic acidosis and neurologic dysfunctions) and Leigh syndrome. PDH has also been implicated in oncogene-induced senescence.[2] PDH measurements can provide insights into metabolic functions and oncogenesis.

This kit provides a simple and direct procedure for measuring pyruvate dehydrogenase activity in a variety of samples. Pyruvate dehydrogenase activity is determined using a coupled enzyme reaction, which results in a colorimetric (450 nm) product proportional to the enzymatic activity present. One unit of pyruvate dehydrogenase is the amount of enzyme that will generate 1.0 μmole of NADH per minute at 37 °C.

### Components

The kit is sufficient for 100 assays in 96 well plates.

| | |
|---|---|
| PDH Assay Buffer<br>Catalog Number MAK183A | 25 mL |
| PDH Substrate<br>Catalog Number MAK183B | 1 vl |
| PDH Developer<br>Catalog Number MAK183C | 1 vl |
| NADH Standard<br>Catalog Number MAK183D | 1 vl |
| PDH Positive Control<br>Catalog Number MAK183E | 10 μL |

### Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader
- Saturated ammonium sulfate (~4.1 M, optional for samples containing small interfering molecules)
- Mitochondria Isolation Kit (optional for mitochondria samples, Catalog Number MITOISO1 for tissue, MITOISO2 for cells, MITOISO3 for yeast, or equivalent)

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

PDH Assay Buffer – Allow buffer to come to room temperature before use. Store at 2–8 °C or –20 °C.

PDH Substrate – Reconstitute with 220 μL of water. Store at –20 °C. Keep on ice while in use. Use within two months.

PDH Developer – Reconstitute with 220 μL of water. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution.

NADH Standard – Reconstitute with 400 μL of water 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 – Reconstitute with 100 µL of PDH Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light at –20 °C. Use within 2 months of reconstitution. Keep on ice while in use.

**Storage/Stability**
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

**Procedure**
All samples and standards should be run in duplicate.

NADH Standards for Colorimetric Detection
Add 0, 2, 4, 6, 8, and 10 µL of the 1.25 mM (1.25 nmole/µL) NADH Standard Solution into a 96 well plate, generating 0 (blank), 2.5, 5, 7.5, 10, and 12.5 nmole/well standards. Add PDH Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid samples can be assayed directly.

Tissue samples (10 mg) or cells ($1 \times 10^6$) can be homogenized in 100 µL of ice-cold PDH Assay Buffer. Keep on ice for 10 minutes. Centrifuge the samples at 10,000 × *g* for 5 minutes to remove insoluble material. Transfer supernatant to fresh tube.

When analyzing PDH activity from mitochondria, it is recommended to isolate the mitochondria from fresh tissue or cells.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

For samples exhibiting significant background, include a Sample Blank for each sample by omitting the PDH Substrate. The Sample Blank readings can then be subtracted from the sample readings.

Add 5–50 µL of sample to duplicate wells. Bring samples to a final volume of 50 µL with PDH Assay Buffer.

For the positive control (optional), add 1–10 µL of the PDH Positive Control solution to wells and adjust to 50 µL with the PDH Assay Buffer.

Note: Small molecules in some tissues such as liver may interfere with the assay. To remove small molecules, it is suggested to use an ammonium sulfate precipitation method. Pipette 50–100 µL of lysate into a fresh tube, add 2× volume of saturated ammonium sulfate ((~4.1 M at room temperature) and keep on ice for 20 minutes. Centrifuge at 10,000 × *g* for 5 minutes, remove and discard the supernatant, and resuspend the pellet to the original volume with PDH Assay Buffer.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the Reaction Mix is required for each reaction (well).

**Table 1.**

Reaction Mixes

| Reagent | Standards and Samples | Sample Blank |
|---|---|---|
| PDH Assay Buffer | 46 µL | 48 µL |
| PDH Developer | 2 µL | 2 µL |
| PDH Substrate | 2 µL | – |

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate at 37 °C. After 2–3 minutes, take the initial measurement. Measure the absorbance at 450 nm [$(A_{450})_{initial}$] at the initial time ($T_{initial}$).
Note: It is essential that $(A_{450})_{initial}$ is in the linear range of the standard curve.

4. Continue to incubate the plate at 37 °C taking measurements ($A_{450}$) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (12.5 nmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final absorbance measurement [$(A_{405})_{final}$] for calculating the enzyme activity would be the penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve (see step 5). The time of the penultimate reading is $T_{final}$.
Note: It is essential that $(A_{450})_{final}$ falls within the linear range of the standard curve.

**Results**

Calculations

Correct for the background by subtracting the final measurement [$(A_{450})_{final}$] obtained for the 0 (blank) NADH Standard from the final measurement [$(A_{450})_{final}$] of the standards and samples. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate NADH Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in absorbance measurement from $T_{initial}$ to $T_{final}$ for the samples.

$$\Delta A_{450} = (A_{450})_{final} - (A_{450})_{initial}$$

Subtract the Sample Blank $\Delta A_{450}$ value from the sample $\Delta A_{450}$ reading to obtain the corrected measurement. Using the corrected measurement, determine the amount of NADH (nmole/well) generated by the PDH assay between $T_{initial}$ and $T_{final}$ ($S_a$).

PDH activity:

$$\text{PDH Activity} = \frac{S_a}{(\text{Reaction Time}) \times S_v}$$

where:

$S_a$ = Amount of NADH (nmole) generated in unknown sample well between $T_{initial}$ and $T_{final}$ from standard curve

Reaction Time = $T_{final} - T_{initial}$ (minutes)

$S_v$ = sample volume (mL) added to well

PDH activity is reported as nmole/min/mL=milliunit/mL.

One unit of pyruvate dehydrogenase is the amount of enzyme that will generate 1.0 μmole of NADH per minute at pH 7.5 at 37 °C.

Sample Calculation:

Amount of NADH ($S_a$) = 5.84 nmole (from standard curve)

($T_{initial}$) = 3 minute

($T_{final}$) = 32 minutes

Sample volume ($S_v$) = 0.05 mL

PDH activity in sample well:

$$\text{nmole/min/mL (milliunits/mL)} = \frac{5.84 \text{ nmole/well}}{(32 \text{ min} - 3 \text{ min}) \times 0.05 \text{ mL/well}} = 4.03$$

**References**


1. Kato, M. et al., Structural basis for inactivation of the human pyruvate dehydrogenase complex by phosphorylation: role of disordered phosphorylation loops. Structure, **16(12)**, 1849–1859 (2008).
2. Kaplon, J. et al., A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. Nature, **498(7452)**, 109–112 (2013).

**Troubleshooting Guide**

| Problem | Possible Cause | Suggested Solution |
|---|---|---|
| Assay not working | Cold assay buffer | Assay Buffer must be at room temperature |
| | Omission of step in procedure | Refer and follow Technical Bulletin precisely |
| | Plate reader at incorrect wavelength | Check filter settings of instrument |
| | Type of 96 well plate used | For colorimetric assays, use clear plates |
| Samples with erratic readings | Samples prepared in different buffer | Use the Assay Buffer provided or refer to Technical Bulletin for instructions |
| | Cell/Tissue culture samples were incompletely homogenized | Repeat the sample homogenization, increasing the length and extent of homogenization step. |
| | Samples used after multiple freeze-thaw cycles | Aliquot and freeze samples if needed to use multiple times |
| | Presence of interfering substance in the sample | If possible, dilute sample further |
| | Use of old or inappropriately stored samples | Use fresh samples and store correctly until use |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Use of expired kit or improperly stored reagents | Check the expiration date and store the components appropriately |
| | Allowing the reagents to sit for extended times on ice | Prepare fresh Reaction Mixes before each use |
| | Incorrect incubation times or temperatures | Refer to Technical Bulletin and verify correct incubation times and temperatures |
| | Incorrect volumes used | Use calibrated pipettes and aliquot correctly |
| Non-linear standard curve | Use of partially thawed components | Thaw and resuspend all components before preparing the Reaction Mixes |
| | Pipetting errors in preparation of standards | Avoid pipetting small volumes |
| | Pipetting errors in the Reaction Mix | Prepare Reaction Mixes whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the plate well |
| | Standard stock is at incorrect concentration | Refer to the standard dilution instructions in the Technical Bulletin |
| | Calculation errors | Recheck calculations after referring to Technical Bulletin |
| | Substituting reagents from older kits/lots | Use fresh components from the same kit |
| Unanticipated results | Samples measured at incorrect wavelength | Check the equipment and filter settings |
| | Samples contain interfering substances | If possible, dilute sample further |
| | Sample readings above/below the linear range | Concentrate or dilute samples so readings are in the linear range |