TECHNICAL MANUAL

Pyruvate Decarboxylase (PDC) Activity Assay Kit

- **SKU CODE:** MAES0260
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Sample preparation
- Add blank and sample
- Add buffer solution and reaction working solution
- Measure OD values at 340 nm

2. Intended use

This kit can be used to measure pyruvate decarboxylase (PDC) activity in serum, plasma, tissue and cell samples.

3. Detection principle

Pyruvate decarboxylase (PDC) mainly exists in yeast and is one of the key enzymes in ethanol fermentation. PDC catalyzes pyruvate decarboxylation to acetaldehyde, which reacts under the action of ethanol dehydrogenase (ADH) and catalyzes NADH to convert into NAD+. NADH has a characteristic absorption peak at 340 nm. The PDC activity can be calculated by measuring the OD value at 340 nm.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	10 mL x 1 vial	20 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 2	Substrate A	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 3	Enzyme Reagent	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Substrate B	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
	UV Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (330-350 nm, optimum wavelength: 340 nm)

Reagents:

Normal saline (0.9% NaCl)

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of substrate A working solution:** Dissolve a vial of substrate A with 5 mL double distilled water. Keep it on ice protected from light for use. Store at -20 °C for 3 days protected from light.
3. **Preparation of enzyme working solution:** Dissolve a vial of enzyme reagent with 1.2 mL double distilled water. Keep it on ice protected from light for use. Store at -20 °C for 7 days protected from light.
4. **Preparation of substrate B working solution:** Dissolve a vial of substrate B with 1.2 mL double distilled water. Keep it on ice protected from light for use. Store at -20 °C for 3 days protected from light.
5. **Preparation of reaction working solution:** Before testing, prepare sufficient reaction working solution according to the test wells. For example, prepare 1500 µL of reaction working solution (mix well 500 µL of substrate A working solution, 500 µL of enzyme working solution, and 500 µL of substrate B working solution). The reaction working solution should be prepared immediately before use.

7. Sample preparation

Sample preparation:

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80 °C for one month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Wash tissue in cold normal saline (0.9% NaCl).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4 °C.

4. Centrifuge at 12,000 xg for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Cells:

1. Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

2. Wash cells with normal saline (0.9% NaCl).

3. Homogenize 1×10^6 cells in 200 µL normal saline (0.9% NaCl) with an ultrasonic cell disruptor at 4 °C.

4. Centrifuge at 12,000 xg for 10 minutes to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample:

The recommended dilution factor for different samples is as follows (for reference only):

10% Mouse liver tissue homogenate: 25-50

10% Mouse kidney tissue homogenate: 25-50

10% Mouse heart tissue homogenate: 25-50

Rat serum: 1

Rabbit plasma: 1

HL-60 cell: 3-5

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

8. The key points of the assay

- All reagents should be stored strictly with light protection and avoid repeated freezing and thawing.
- The change of OD per unit time (min) should be controlled within 0.2.

9. Operating steps

1. Blank well: Add 20 µL of double distilled water to the wells. Sample well: Add 20 µL of sample to the wells.

2. Add 120 µL of buffer solution into each well.

3. Add 60 µL of reaction working solution into each well.

4. Mix thoroughly, measure the OD value of each well at 1 min and 3 min respectively at 340 nm with microplate reader, recorded as A1, A2, ΔA = A1 - A2.

10. Calculation

The sample:

1. Serum (plasma) sample:

Definition: The amount of PDC in 1 L liquid sample per 1 minute that hydrolyzes the NADH to produce 1 μmol NAD at 25 °C is defined as 1 unit.

PDC activity (U/L) = (ΔA_sample - ΔA_blank) ÷ (ε × d) ÷ T × f × 10^6

2. Tissue and cell sample:

Definition: The amount of PDC in 1 g tissue or cell protein per 1 minute that hydrolyzes the NADH to produce 1 μmol NAD at 25 °C is defined as 1 unit.

PDC activity (U/g_{prot}) = (ΔA_sample - ΔA_blank) ÷ (ε × d) ÷ C_{pr} ÷ T × f × 10^6

[Note]

ΔA_sample: The change OD value of sample well, A_1-A_2.

ΔA_blank: The change OD value of blank well, A_1-A_2.

ε: Molar extinction coefficient of NADH, 6.22×10^3 L/(mol•cm).

d: The optical path of microplate, 0.6 cm.

C_{pr}: Concentration of protein in sample, g_{prot}/L.

f: Dilution factor of sample before testing.

T: The reaction time, 2 min.

10^6: 1 mol = 10^6 μmol.

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.70	13.50	22.60
%CV	3.2	2.7	2.5

Inter-assay Precision

Three human serum samples were assayed 17 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	2.70	13.50	22.60
%CV	2.8	3.1	3.4

Recovery

Three samples of high concentration, middle concentration and low concentration were tested in parallel 6 times for each concentration to obtain an average recovery rate of 105%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	7.4	19.3	25.5
Observed Conc. (U/L)	7.8	20.7	26.0
Recovery rate (%)	106	107	102

Sensitivity

The analytical sensitivity of the assay is 0.67 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

12. Appendix II Example Analysis

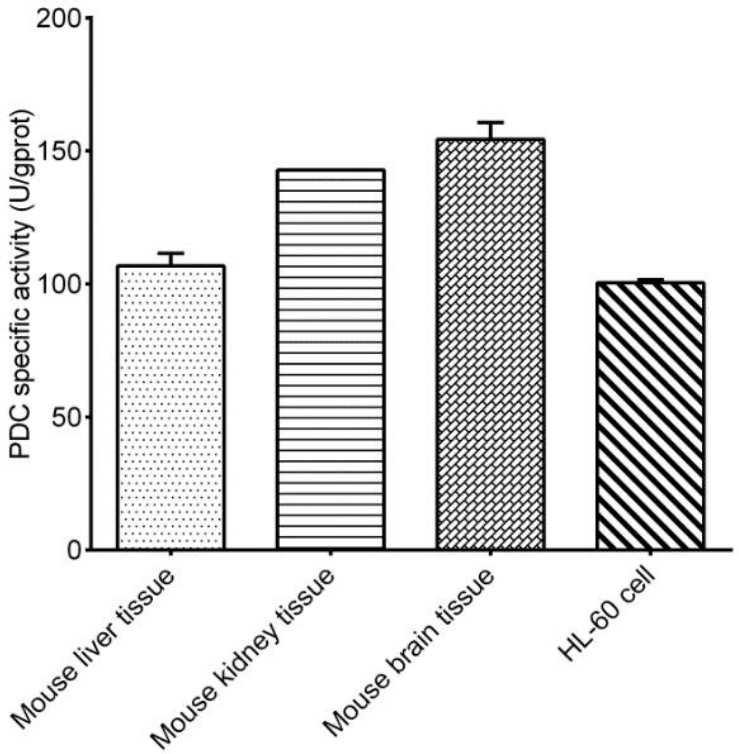
Example analysis:

For 10% mouse liver tissue homogenate, diluted 50 times, and carried out according to the operation steps. The results are as follows:

The A_1 of the blank well is 1.054, the A_2 of the blank well is 1.053, the A_1 of the sample well is 0.674, the A_2 of the sample well is 0.448, the concentration of protein in sample is 14.18 g_{prot}/L, and the calculation result is:

PDC activity (U/g_{prot}) = ((0.674-0.448)-(1.054-1.053)) ÷ (6220 × 0.6) ÷ 14.18 ÷ 2 × 50 × 10^6 = 106.29 U/g_{prot}

Detect 10% mouse liver tissue homogenate (the concentration of protein is 14.18 g_{prot}/L, diluted 50 times), 10% mouse kidney tissue homogenate (the concentration of protein is 13.37 g_{prot}/L, diluted 50 times), 10% mouse brain tissue homogenate (the concentration of protein is 5.99 g_{prot}/L, diluted 50 times), and HL-60 cell (the concentration of protein is 5.99 g_{prot}/L, diluted 3 times) according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.
4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.