TECHNICAL MANUAL

Proline Dehydrogenase (ProDH) Activity Assay Kit

- **SKU CODE:** MAES0478
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can be used to measure proline dehydrogenase (ProDH) activity in tissue samples.

2. Detection principle

The hydrogen produced by proline during the catalytic process of ProDH can reduce the chromogenic agent through an electron acceptor, resulting in color reduction. The ProDH activity can be characterized by measuring the rate of absorbance decrease at 600 nm.

3. Kit components & storage

Item	Component	Size 1 (48T)	Size 2 (96T)	Storage
Reagent 1	Extract Solution A	55 mL x 1 vial	55 mL x 2 vials	-20 °C, 12 months
Reagent 2	Extract Solution B	1 mL x 1 vial	1 mL x 2 vials	-20 °C, 12 months, shading light
Reagent 3	Chromogenic Agent	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 4	Substrate	0.1 mL x 1 vial	0.2 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 5	Reaction Solution	10 mL x 1 vial	20 mL x 1 vial	-20 °C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

4. Materials prepared by users

Instruments:

Microplate reader (580-620 nm, optimum wavelength: 600 nm), Centrifuge, Ultrasound instrument (20% power)

5. Reagent preparation

1. Keep Extract Solution B and Substrate on ice and protected from light during use. Equilibrate other reagents to 25 °C before use. The substrate can be aliquoted and stored at -20 °C protected from light.
2. **Preparation of chromogenic solution:** Dissolve one vial of Chromogenic Agent with 10 mL of double distilled water. Dissolve by ultrasonication for 10 minutes. Store at -20 °C for 5 days protected from light.
3. **Preparation of chromogenic working solution:** Before testing, prepare sufficient chromogenic working solution according to the number of test wells. For example, prepare 1880 µL of chromogenic working solution (mix well 1380 µL of Reaction Solution and 500 µL of chromogenic solution). Store at 2-8 °C for 2 days protected from light. (Ultrasonicate for 10 minutes protected from light before use).
4. **Preparation of reaction working solution:** Before testing, prepare sufficient reaction working solution according to the number of test wells. For example, prepare 1505 µL of reaction working solution (mix well 1500 µL of chromogenic working solution and 5 µL of Substrate). The reaction working solution should be prepared fresh.

6. Sample preparation

1. **Sample preparation - Tissue sample:** ① Harvest the amount of tissue needed for each assay (initial recommendation 50 mg). ② Wash tissue in cold PBS (0.01 M, pH 7.4). ③ Homogenize 50 mg tissue in 445 µL Extract Solution A and 5 µL Extract Solution B with a dounce homogenizer at 4 °C. ④ Centrifuge at 10,000 x g for 10 minutes at 4 °C to remove insoluble material. Collect supernatant and keep it on ice for detection. ⑤ Determine the protein concentration of plant tissue supernatant (MAES0126). Protein concentration is not measured in animal tissues.
2. **Dilution of sample:** The recommended dilution factor for different samples is as follows (for reference only): 10% Green leaves tissue homogenization: 1 10% Mouse lung tissue homogenization: 1 10% Garlic tissue homogenization: 1 Note: The diluent is Extract Solution A. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

7. The key points of the assay

Ensure complete dissolution of the Chromogenic Agent in double distilled water using an ultrasound instrument or vortex mixer.

8. Operating steps

1. Blank wells: Add 40 μL of double distilled water to the corresponding wells; Sample wells: Add 40 μL of sample to the corresponding wells.
2. Add 200 μL of reaction working solution to each well.
3. Mix fully with microplate reader for 5 seconds, incubate at 37 °C for 15 minutes and measure the OD value (A1) of each well at 600 nm.
4. Incubate at 37 °C for 60 minutes and mix fully with microplate reader for 5 seconds, measure the OD value (A2) of each well at 600 nm.

9. Calculation

The sample:

1. Plant sample:

Definition: The amount of enzyme in 1 g tissue protein that hydrolyzes 1 mmol DCPIP in 1 min at 37 °C is defined as 1 unit.

ProDH activity (U/gprot) = (ΔA_sample - ΔA_blank) × V_1 × f ÷ ε ÷ d ÷ V_2 ÷ T ÷ C_{pr} × 1000*

2. Animal sample:

Definition: The amount of enzyme in 1 g tissue that hydrolyzes 1 mmol DCPIP in 1 min at 37 °C is defined as 1 unit.

ProDH activity (U/g wet weight) = (ΔA_sample - ΔA_blank) × V_1 × f ÷ ε ÷ d ÷ V_2 ÷ T ÷ W × 1000*

[Note]

ΔA_sample: ΔA_sample = A_1 - A_2.

ΔA_blank: ΔA_blank = A_1 - A_2.

ε: The molar extinction coefficient at 600 nm, 18.7 L/mol/cm.

d: Optical path, 0.6 cm.

T: Reaction time, 60 min.

V_1: The volume of reaction system, 0.24 mL.

V_2: The volume of sample added to the reaction system, 0.04 mL.

f: Dilution factor of sample before test.

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

W: The wet weight of sample, 0.1 g.

1000*: 1 mol/L = 1000 mmol/L.

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three green leaves 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)	0.20	0.80	1.00
%CV	3.0	3.3	3.9

Inter-assay Precision

Three green leaves samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.20	0.80	1.00
%CV	8.9	9.6	10.0

Recovery

Three samples of high concentration, middle concentration and low concentration were tested with each concentration analyzed 6 times in parallel to obtain an average recovery rate of 97%.

Recovery

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.2	0.8	1

	Sample 1	Sample 2	Sample 3
Observed Conc. (U/L)	0.2	0.8	1.0
Recovery rate (%)	95	100	96

Sensitivity

The analytical sensitivity of the assay is 0.04 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.

11. Appendix II Example Analysis

Example analysis:

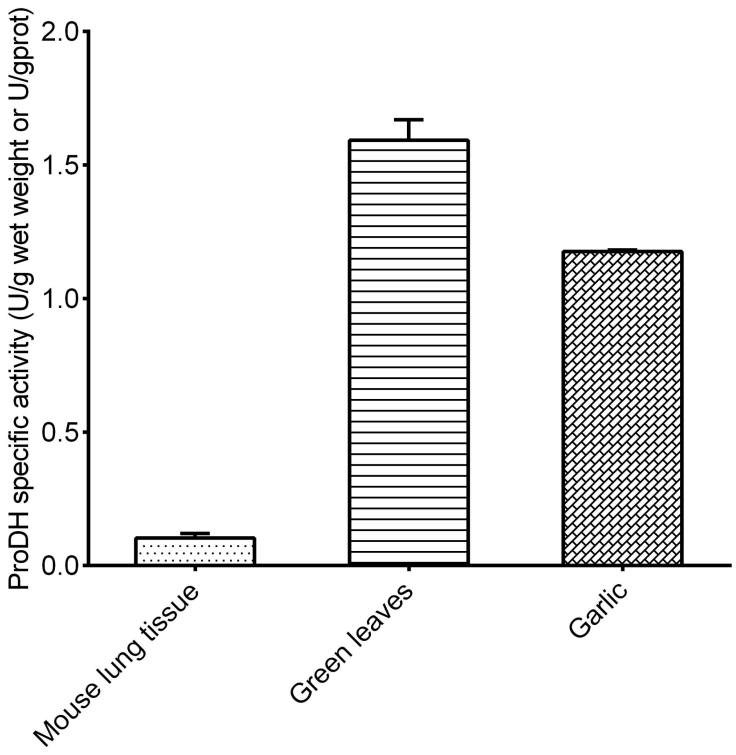
Take 40 µL of 10% green leaves tissue homogenization and carry out the assay according to the operating steps. The results are as follows:

The A_1 of the blank is 0.604, the A_2 of the blank is 0.587, ΔA_blank = 0.604 - 0.587 = 0.017. The A_1 of the sample is 0.679, the A_2 of the sample is 0.610, ΔA_sample = 0.679 - 0.610 = 0.069, and the calculation result is:

ProDH activity (U/gprot) = (0.069 – 0.017) × 0.24 ÷ 18.7 ÷ 0.6 ÷ 0.04 ÷ 60 ÷ 0.29 × 1000

= 1.60 U/gprot

Detect 10% mouse lung tissue homogenization, 10% green leaves tissue homogenization (the concentration of protein in sample is 0.29 gprot/L) and 10% garlic tissue homogenization (the concentration of protein in sample is 0.87 gprot/L) according to the protocol, the result is as follows:



12. 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. Please follow the instructions strictly during the experiments.

3. Protection methods must be taken by wearing a lab coat and latex gloves.

4. If the concentration of the substance is not within the detection range exactly, additional dilution or concentration should be performed on the sample.

5. It is recommended to perform a pre-test if your sample is not listed in the instruction manual.

6. The experimental results are closely related to reagent quality, operations, environmental conditions and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption caused by using the assay kits. It is advisable to calculate the possible sample usage and reserve sufficient samples before use.

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.