（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K860-M

Specification：48T(46 samples)/ 96T(94 samples)

Measuring instrument：Microplate reader (580-620 nm)

Detection range：0.04-1.10 U/L

Elabscience® Proline Dehydrogenase (ProDH) Activity Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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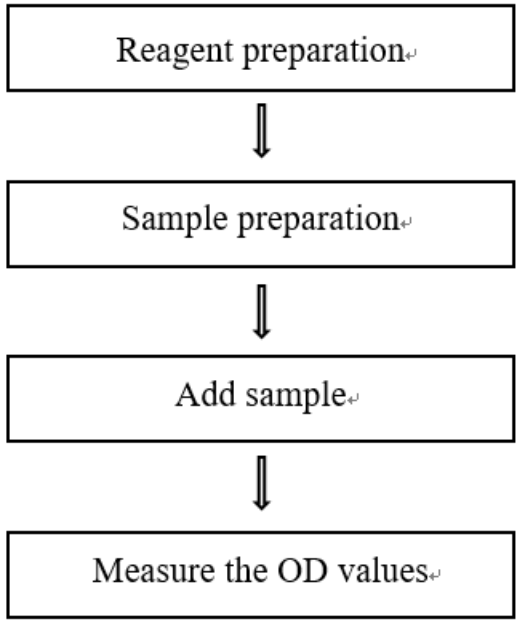
Assay summary



Intended use

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

Detection principle

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

Kit components & storage

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

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

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

Reagent preparation

① Keep extract solution B and substrate on ice protected from light during use. Equilibrate other reagents to 25℃ before use. The substrate can be aliquoted storage at -20 ℃ protected from light.

② The preparation of chromogenic solution：
Dissolve one vial of chromogenic agent with 10 mL of double distilled water, dissolved by ultrasound for 10 min. Store at -20 ℃ for 5 days protected from light.

③ The preparation of chromogenic working solution：
Before testing, please prepare sufficient chromogenic working solution according to the 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℃ for 2 days protected from light. (Ultrasound 10 min protected from light before use).

④ The preparation of reaction working solution：
Before testing, please prepare sufficient reaction working solution according to the 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 on spot.

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℃.

④ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Determine the protein concentration of plant tissue supernatant (E-BC-K168-M). 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):

Sample type	Dilution factor
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 do pretest to confirm the dilution factor.

The key points of the assay

Complete dissolution of chromogenic agent in double distilled water was ensured by ultrasound instrument or vortex mixer.

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 s, incubate at 37℃ for 15 min and measure the OD value (A_1) of each well at 600 nm.

④ Incubate at 37℃ for 60 min and mix fully with microplate reader for 5 s, measure the OD value (A_2) of each well at 600 nm.

Calculation

The sample:

1. Plant sample:

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

$$\text{ProDH activity (U/gprot)} = (\Delta A_{sample} - \Delta A_{blank}) \times V_1 \times f \div \varepsilon \div d \div V_2 \div T \div C_{pr} \times 1000^*$$

2. Animal sample:

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

$$\text{ProDH activity (U/g wet weight)} = (\Delta A_{sample} - \Delta A_{blank}) \times V_1 \times f \div \varepsilon \div d \div V_2 \div T \div W \times 1000^*$$

[Note]

ΔA_{sample}: $\Delta A_{sample} = A_1 - A_2$.

ΔA_{blank}: $\Delta A_{blank} = A_1 - A_2$.

ε: The molar extinction coefficient of 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 of sample, 0.1g.

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

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

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

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.

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

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 97%.

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

Appendix II Example Analysis

Example analysis:

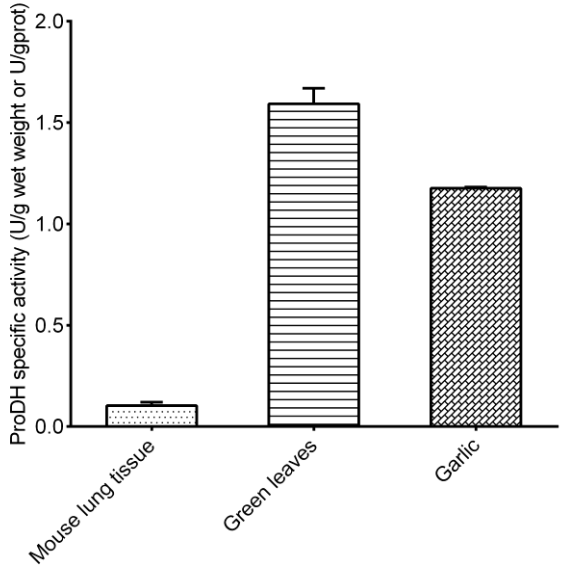
Take 40 μL of 10% green leaves tissue homogenization, carry the assay according to the operation steps. The results are as follows:

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:

$$\text{ProDH activity (U/gprot)} = (0.069 - 0.017) \times 0.24 \div 18.7 \div 0.6 \div 0.04 \div 60 \div 0.29 \times 1000$$

$$= 1.60 \text{ U/gprot}$$

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:



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.