Proline Dehydrogenase (ProDH) Assay Kit

Catalog No.: abx294435

Size: 96 tests

Detection Range: 0.04 U/L – 1.10 U/L

Storage: Store reagents in the dark at -20°C for up to 12 months.

Application: For detection and quantification of proline dehydrogenase (ProDH) activity in tissue samples.

Introduction

Abbexa's Proline Dehydrogenase Assay Kit is a quick, convenient, and sensitive method for measuring and calculating ProDH activity. The reaction catalyzed by ProDH will generate hydrogen, which will reduce the Chromogenic Reagent. This causes the color to lighten. The color change should be monitored by measuring the rate of absorbance decrease at 600 nm. The rate of absorbance decrease is proportional to the ProDH activity.

Kit components

1. 96-well microplate
2. Substrate: 0.2 ml
3. Extraction Solution A: 2 x 55 ml
4. Extraction Solution B: 2 x 1 ml
5. Chromogenic Reagent: 2 vials
6. Assay Buffer: 20 ml
7. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (580 nm – 620 nm)
2. Pipette and pipette tips
3. Vials/tubes
4. Ultrasound instrument (20% power)
5. Centrifuge

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Tissue Homogenates:** Weigh 50 mg of tissue and wash with pre-chilled PBS (0.01 M, pH 7.4). For each 50 mg of tissue, add 445 µl of Extraction Solution A and 5 µl of Extraction Solution B. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 10,000 x g at 4°C for 10 min to remove insoluble material. Collect the supernatant and keep it on ice. Protein concentration in the plant tissue extract should be determined separately.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Diluent A, then carry out the assay procedure.

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

Sample Type	Dilution Factor
10% Leaf Tissue Homogenate	1
10% Mouse Lung Tissue Homogenate	1
10% Garlic Tissue Homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

All reagents except Extraction Solution B and the Substrate should be equilibrated to room temperature before use.

- **Extraction Solution B:** Keep on ice in the dark throughout the experiment. It is recommended to aliquot the Substrate for storage at -20°C.
- **Chromogenic Reagent Stock Solution:** Dissolve one vial of Chromogenic Reagent with 10 ml of double distilled water, and ultrasonicate for 10 minutes to reconstitute. The reconstituted Chromogenic Reagent Stock Solution can be stored at -20°C in the dark for up to 5 days.
- **Chromogenic Reagent Working Solution:** Prepare enough Chromogenic Reagent Working Solution for the number of wells being assayed. For example, to prepare 1880 µl of Chromogenic Reagent Working Solution, mix 1380 µl of Assay Buffer and 500 µl of reconstituted Chromogenic Reagent Stock Solution. The Chromogenic Reagent Working Solution can be stored at 2-8°C in the dark for up to 2 days, and should be ultrasonicated in the dark for 10 minutes before use in the assay.
- **Reaction Working Solution:** Prepare enough Reaction Working Solution for the number of wells being assayed. For example, to prepare 1505 µl of Reaction Working Solution, mix 1500 µl of Chromogenic Reagent Working Solution and 5 µl of Substrate. The Reaction Working Solution should be prepared immediately before use.

B. Assay Procedure

1. Add 40 µl of double distilled water to the blank wells.
2. Add 40 µl of sample to the sample wells.
3. Add 200 µl of Reaction Working Solution to each well.
4. Mix thoroughly with a microplate reader for 5 seconds.
5. Incubate at 37°C for 15 min and measure the OD value (A_1) of each well at 600 nm.
6. Incubate at 37°C for 60 min, mix for 5 seconds using a microplate reader and measure the OD value (A_2) of each well at 600 nm.

C. Calculation of Results

1. Plant tissue samples:

1 unit of enzyme is defined as the amount of enzyme in 1 g of tissue protein that hydrolyses 1 mmol of DCPIP in 1 minute at 37°C.

$$\text{ProDH activity}\left(\text{U/g}_{\text{prot}}\right)=\frac{\left(\Delta A_{\text{sample}}-\Delta A_{\text{blank}}\right)\times V_1\times f\times 1000}{\varepsilon\times d\times V_2\times T\times C_p}$$

2. Animal tissue samples:

1 unit of enzyme is defined as the amount of enzyme in 1 g of tissue that hydrolyses 1 mmol of DCPIP in 1 minute at 37°C.

$$\text{ProDH activity}\left(\text{U}/g_{wet\ weight}\right) = \frac{\left(\Delta A_{sample} - \Delta A_{blank}\right) \times V_{reaction} \times f \times 1000}{\varepsilon \times d \times V_{sample} \times T \times W}$$

where:

ΔA_{sample}	$\Delta A_{sample} = \Delta A_1 - \Delta A_2$
ΔA_{blank}	$\Delta A_{blank} = \Delta A_1 - \Delta A_2$
ε	Molar extinction coefficient at 600 nm, 18.7 L/mol/cm
d	Optical path, 0.6 cm
T	Reaction time, 60 min
$V_{reaction}$	Volume of reaction system, 0.24 ml.
V_{sample}	Volume of sample added to reaction system, 0.04 ml
C_P	Concentration of protein in sample g/L
W	Wet mass of sample, 0.1 g
1000	Unit conversion: 1 L = 1000 ml
f	The dilution factor of sample

D. Technical Support

For troubleshooting and technical assistance, please contact us at support@abbexa.com.