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

Proline (Pro) Assay Kit

Catalog No.: BC00038

Size: 100T

Please read the instructions carefully before use. If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉ Email (Sale) order@enkilife.com

✉ Email (Techsupport) techsupport@enkilife.com

☎ Tel: 0086-27-87002838

Website: www.enkilife.com

Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Proline (Pro) Assay Kit
Detection Method	Colorimetric
Sample Type	Plant tissue, Honey, etc.
Assay Type	Quantitative
Detection Instrument	Microplate reader (520 nm)

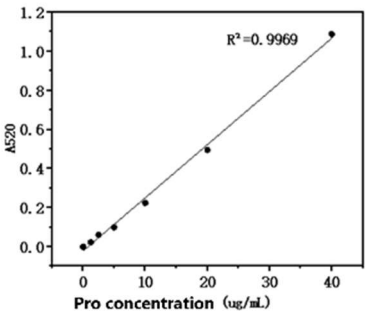
Product Introduction

In organisms, proline is not only an ideal osmotic regulating substance, but also can be used as a protective substance for membranes and enzymes and a free radical scavenger, thereby protecting the growth of plants under osmotic stress. For the accumulation of potassium ions, another important osmotic regulating substance in the body, in the vacuole, proline can also regulate the osmotic balance of the cytoplasm.

Principle

Among the amino acids in plants, only proline (Pro) can react with acidic ninhydrin to generate a stable red product (as shown below). This product has a maximum absorption peak at a wavelength of 520nm, and its absorption value is linearly related to the proline content. Therefore, the proline content in the sample can be determined by the acidic ninhydrin method.
The following standard curve is for reference only:

Pro concentration (μg/mL)	A520
0	0
1.25	0.023
2.5	0.061
5	0.099
10	0.224
20	0.496
40	1.088



y = 0.0272x + 0.022 (R^2=0.9969)

Components

Serial number	Components	Size(100T)	Storage
Reagent 1	Extraction buffer	50mLx1 bottle	-20℃, store at 2-8°C away from light after opening.

Reagent 2	Ninhydrin	1.5g x1 bottle	-20℃, store at 2-8°C away from light after opening.
Reagent 3	Acid reagent	20 ml x1 bottle	-20℃, store at 2-8°C away from light after opening.
Reagent 4	400μg/mL standard	1ml x1 tube	-20℃, store at 2-8°C away from light after opening.
Consumable 1	Microplate(96 wells)	1 plate	RT
Consumable 2	Plate Sealer	2 pieces	RT

Storage

The unopened test kit can be stored at -20℃ for 12 months. After opening, it can be stored at 2-8℃ away from light for 6 months.

Experimential Preparation

- Sample processing

1. Sample processing

Honey sample: Take fresh honey (0.020-1g), weigh it, put it into a test tube, add reagent 1 at a ratio of weight (g): volume (mL) = 1:10, extract it in a boiling water bath for 15 min (shake the test tube to extract), take it out and cool it to room temperature, centrifuge it at 4℃, 10000xg for 15min, and take the supernatant for testing.

Tissue sample: Take 0.020-1.0g of fresh tissue block, rinse with double distilled water, absorb with filter paper, weigh, put into a homogenization container, add reagent 1 at a ratio of weight (g): volume (mL) = 1:9, homogenize, centrifuge at 4℃, 10000×g for 15min, and take the supernatant for testing.

2. Dilution of samples

Before the formal test, it is necessary to select 2-3 samples with large expected differences and dilute them into different concentrations for preliminary experiments. According to the results of the preliminary experiments and the linear range of this kit: 1.25-40μg/mL, you can refer to the following table for dilution (for reference only):

Sample	Dilution multiple	Sample	Dilution multiple
Pepper	10-30	Orange peel/pulp	10-20
Carrot	No dilution	Lettuce	No dilution
Pothos	No dilution	Grape	No dilution
Garlic	No dilution	Yellow peach	No dilution
Pear	No dilution	Plum	No dilution

Note: The diluent is Reagent 1.

- Preparation of the assay kit

Take out all reagents and return them to room temperature before use. **You must prepare acetic acid yourself** .

1. Preparation of reaction working solution: Mix reagent 2 (g): acetic acid (mL): reagent 3 (mL) in a ratio of 1:24:16, shake and heat to dissolve (<70°C), cool and store at room temperature away from light, and use immediately after preparation.
2. Dilution of different concentrations of standards: dilute the given concentration of 400 μg/mL of reagent 4 with reagent 1 to a standard concentration of 40 μg/mL and dilute it in half to obtain standards with concentrations of 40, 20, 10, 5, 2.5, and 0 (blank well) μg/mL for use.

Operation process

1. Standard wells: Take 0.5 mL of standard samples of different concentrations and add them into 5 mL glass test tubes.
2. Assay well: Take 0.5mL of the samples and add it into a 5mL glass test tube.
3. Add 0.5 mL of acetic acid to each well in step (1) in turn.
4. Add 0.5 mL of reaction working solution to each well in step (2) and vortex to mix.
5. Tie the mouth of the test tube tightly with plastic wrap, make a small hole in the plastic wrap, and place it in a boiling water bath for 30 min.
6. The running water was cooled to room temperature, and after each tube was fully mixed, 200 μL was taken and added to each corresponding well of the ELISA plate.
7. The OD value of each well was measured at 520 nm on an enzyme reader.
8. Calculate the sample change OD value ΔA520 = A sample - A blank (blank is when the concentration of the standard is 0)

	Standard	**Sample**
Different concentrations of standard products (mL)	0.5	--
Sample to be tested (mL)	--	0.5
Acetic acid (mL)	0.5	0.5
Reaction working solution (mL)	0.5	0.5

Result calculation

Standard fitting curve: y = ax + b

Concentration calculation formula: GOD activity (U/mL) = (ΔA520- b) ÷ a× f

Annotation:

y: OD value of standard well - OD value of blank well (OD value when the concentration of standard is 0)

x: concentration corresponding to the absorbance

a: slope of the curve
b: intercept of the curve
ΔA 520 : Sample OD value - blank OD value (OD value when the standard concentration is 0)
f: dilution factor of the sample before adding it to the detection system

Notes

1. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
2. Please wear lab coats and latex gloves for protection during the experiment.
3. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.
4. If the sample being tested is not among the sample types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.
5. The final experimental results are closely related to the effectiveness of the reagents, the relevant operations of the experimenter, the experimental environment and other factors. Our company is only responsible for the kit itself, not for the sample consumption caused by the use of the kit. Please fully consider the possible usage of the sample before use and reserve sufficient samples.
6. This product is for Research Use Only, and shall not be used for clinical diagnosis or treatment, food or medicine, or stored in ordinary residences.