

PRODUCT INFORMATION & MANUAL

Proline (Pro) Assay Kit (Colorimetric) NBP3-25781

For research use only.

Not for diagnostic or therapeutic procedures.

www.novusbio.com - P: 303.730.1950 - P: 888.506.6887 - F: 303.730.1966 - technical@novusbio.com

Novus kits are guaranteed for 6 months from date of receipt

Proline (Pro) Colorimetric Assay Kit

Catalog No: NBP3-25781

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 samples without

duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 0.17 µg/mL

Detection range: 0.17-35 µg/mL

Average intra-assay CV (%): 4.5

Average inter-assay CV (%): 5.4

Average recovery rate (%): 105

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲ Intended use

This kit can be used to measure the Proline (Pro) content in plant tissue and honey samples.

▲ Background

Proline is a proteinogenic amino acid that is used in the biosynthesis of proteins. It contains an α -amino group, an α -carboxylic acid group (which is in the deprotonated $\neg COO \neg$ form under biological conditions), and a side chain pyrrolidine, classifying it as a nonpolar (at physiological pH), aliphatic amino acid. It is non-essential in humans, meaning the body can synthesize it from the non-essential amino acid L-glutamate. Proline is the only proteinogenic amino acid with a secondary amine, in that the alpha-amino group is attached directly to the side chain, making the α carbon a direct substituent of the side chain.

▲ Detection principle

Proline can react with acidic-ninhydrin to form stable red compound. The maximum absorption peak of the compound is at 520 nm. And the concentration of Pro can be calculated by measuring the OD value at 520 nm.

▲ Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Extracting Solution	60 mL × 5 vials	2-8°C, 12 months, shading light
Reagent 2	Ninhydrin	6 g × 1 vial	2-8°C , 12 months, shading light
Reagent 3	Acid Reagent	50 mL × 2 vials	2-8°C , 12 months
Reagent 4	100 μg/mL Proline Standard	2 mL × 1 vial	2-8°C , 12 months, shading light

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.

▲ Materials prepared by users



₫ Instruments

Spectrophotometer (520 nm), Water bath, Micropipettor, Vortex mixer, Balance, Centrifuge

Reagents:

Double distilled water, Acetic acid, Methylbenzene, Absolute ethyl alcohol

▲ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

- 1. Please operate in fuming hood. Methylbenzene is poisonous, please take safety precautions.
- 2. Don't wash the cuvette with double distilled water when the cuvette have washed by absolute ethyl alcohol. Because H₂O and methylbenzene are not mutually soluble, it will affect the result.
- 3. The chromogenic agent must be fresh prepared.

Pre-assay preparation

▲ Reagent preparation

- 1. Preparation of chromogenic agent Mix the reagent 2 (g), acetic acid (self-prepared) (mL) and reagent 3 (mL) at a ratio of 1: 24: 16. Heat with agitation until it is dissolved completely (<70°C). Store the solution with shading light after cooling. Prepare the fresh solution before use.
- 10 μg/mL standard application solution
 Dilute the 100 μg/mL proline standard with reagent 1 at a ratio of 1:9 before use.

▲ Sample preparation

1. Honey sample

Weigh 0.02-1 g of honey to the tube, add reagent 1 at a ratio of sample (g) : Volume (mL)= 1: 10. Incubate in 100° C water bath for 15 min (shake the tube constantly), then cool to room temperature, centrifuge at 10000 g for 15 min at 4° C, collect the supernatant for detection.

2. Tissue sample

Weigh 0.02-1 g of fresh tissue and wash with double distilled water for several times, then absorb with filter paper and weigh accurately, add reagent 1 at a ratio of tissue (g): reagent 1 (mL) = 1: 10. Homogenized mechanically with a homogenizer. Then centrifuge at 10000 g for 15 min at 4°C , collect the supernatant for detection.

[Note]: If the sample can't be tested on the day, it can be stored at -80°C for a month.

▲ Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (0.17-35 µg/mL).

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

Sample type	Dilution factor
Green pepper tissue homogenization	1
Carrot tissue homogenization	1
Epipremnum aureum tissue homogenization	1
Lettuce leaf tissue homogenization	1
Honey	1
Cabbages tissue homogenization	1

Note: The diluent is reagent 1.

Assay protocol

▲ Detailed operating steps

- Blank tube: Take 2 mL of reagent 1 into the 10 mL glass test tube.
 Standard tube: Take 2 mL of 10 μg/mL standard application solution into the 10 mL glass test tube.
 - Sample tube: Take 2 mL of sample into the 10 mL glass test tube.
- 2. Add 2 mL of acetic acid (self-prepared), 2 mL of chromogenic agent into the tubes of Step 1, and mix fully with a vortex mixer.
- 3. Fasten the mouth of the tube with plastic film, prick a small hole with a needle. Incubate in 100°C water bath for 30 min, then cool with running water.
- 4. Add 4 mL of methylbenzene (self-prepared), mix fully with a vortex mixer and stand for 10 min.
- Take 3 mL of supernatant to another centrifuge tube, centrifuge at 2325 g for 10 min.
- 6. Collect upper red methylbenzene layer. Set the spectrophotometer to zero with methylbenzene and measure the OD value of each tube at 520 nm with 1 cm optical path quartz cuvette. (Note: Don't wash the cuvette with double distilled water when the cuvette have washed by absolute ethyl alcohol. Because H₂O and methylbenzene are not mutually soluble, it will affect the result.)

The second

▲ Summary operation table

	Blank tube	Standard tube	Sample tube
Reagent 1 (mL)	2		
10 µg/mLstandard application solution (mL)		2	
Sample (mL)			2
Acetic acid (self-prepared) (mL)	2	2	2
Chromogenic agent (mL)	2	2	2

Mix fully. Fasten the mouth of the tube with plastic film, prick a small hole with a needle. Incubate in 100°C water bath for 30 min, then cool with running water. Add 4 mL of methylbenzene (self-prepared), mix fully and stand for 10 min. Take 3 mL of supernatant to another centrifuge tube, centrifuge for 10 min. Collect upper red methylbenzene layer. Set the spectrophotometer to zero with methylbenzene and measure the OD value at 520 nm.

▲ Calculation

Pro content (µg/g) =
$$\frac{\Delta A_1}{\Delta A_2}$$
 × c × V ÷ W × f

Note:

 ΔA_1 : $OD_{Sample} - OD_{Blank}$.

 $\Delta A_2 : OD_{Standard} - OD_{Blank}.$

c: Concentration of standard, 10 $\mu g/mL$.

V: The volume of Reagent 1 for sample preparation.

W: The weight of sample.

f: Dilution factor of sample before test.

Appendix I Data

▲ Example analysis

Weight 0.5 g of daucus carota and cut into pieces, add 5 mL of reagent 1, homogenized the sample, centrifuge at 10000 g for 15 min at 4° C and dilute the supernatant with Reagent 1 for 2.5 times, then take 2 mL of diluted supernatant and carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.065, the average OD value of the blank is 0.004, the average OD value of the standard is 0.444, and the calculation result is:

Pro concentration(μ g/g) = (0.065-0.004)÷(0.444-0.004)× 10 μ g/mL × 5 mL ÷ 0.5 g × 2.5 = 34.66 μ g/g

Appendix II References

- 1. Szabados L, Savoure A. Proline: a multifunctional amino acid. Trends in Plant Science, 2010, 15(2): 89-97.
- 2. Bhaskara G B, Yang T H, Verslues P E. Dynamic proline metabolism: importance and regulation in water limited environments. Frontiers in Plant Science, 2015, 6(6): 789-791.
- 3. Mmf M, Ali E F. Evaluation of proline functions in saline conditions. Phytochemistry, 2017, 140: 52-68.
- 4. Rejeb K B, Abdelly C, Savoure A. How reactive oxygen species and proline face stress together. Plant Physiology & Biochemistry, 2014, 80: 278-284.