TECHNICAL MANUAL

Proline (Pro) Colorimetric Assay Kit

- **SKU CODE:** MAES0282
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

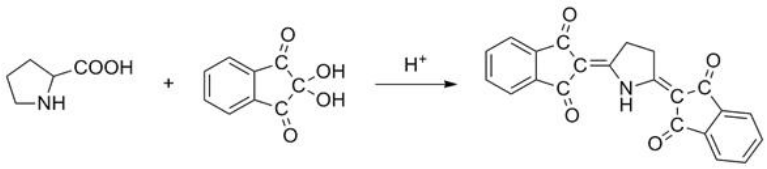
- Reagent preparation
- Sample preparation
- Operating steps
- Measure OD value
- Calculation

2. Intended use

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

3. Detection principle

Proline reacts with acidic ninhydrin to form a stable red compound with a maximum absorption peak at 520 nm. The concentration of proline can be calculated by measuring the optical density value at 520 nm.



4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	50 mL × 3 vials	60 mL × 5 vials	2-8°C, 12 months, shading light
Reagent 2	Ninhydrin	3 g × 1 vial	6 g × 1 vial	2-8°C, 12 months, shading light
Reagent 3	Acid Reagent	50 mL × 1 vial	50 mL × 2 vials	2-8°C, 12 months
Reagent 4	100 μg/mL Proline Standard	1 mL × 1 vial	2 mL × 1 vial	2-8°C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (510-530 nm, optimal wavelength: 520 nm), vortex mixer, water bath, centrifuge.

Reagents:

Double distilled water, acetic acid

6. Reagent preparation

1. Equilibrate all reagents to room temperature before use.
2. **Preparation of reaction working solution:** For each well, prepare 2 mL of reaction working solution by mixing 50 mg of ninhydrin, 1200 µL of acetic acid, and 800 µL of acid reagent. Heat with agitation until completely dissolved (<70°C). Store the solution protected from light after cooling. The reaction working solution should be prepared fresh.
3. **Preparation of control working solution:** For each well, prepare 2 mL of control working solution by mixing 1200 µL of acetic acid (self-prepared) and 800 µL of acid reagent. Store the solution protected from light. The control working solution should be prepared fresh.
4. **Preparation of 10 µg/mL standard solution:** For each well, prepare 2 mL of 10 µg/mL standard solution by mixing 200 µL of 100 µg/mL proline standard with 1800 µL of extracting solution. The 10 µg/mL standard solution should be prepared fresh.

7. Sample preparation

1. **Sample preparation:** Honey sample: 1. Harvest the amount of honey needed for each assay (initial recommendation: 600 mg). 2. Mix 600 mg honey in 6 mL extracting solution. 3. Incubate in 100°C water bath for 15 min (shake the tube constantly). 4. Cool to room temperature, centrifuge at 10,000 × g for 15 min at 4°C, and collect the supernatant for detection. Tissue samples: 1. Harvest the amount of tissue needed for each assay (initial recommendation: 600 mg). 2. Wash tissue in cold PBS (0.01 M, pH 7.4). 3. Homogenize 600 mg tissue in 6 mL extracting solution with a dounce homogenizer at 4°C. 4. Centrifuge at 10,000 × g for 15 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

2. **Dilution of sample:** The recommended dilution factors for different samples are as follows (for reference only): Green pepper tissue homogenate: 10-30 Orange pulp tissue homogenate: 10-20 Epipremnum aureum tissue homogenate: 1 Carrot tissue homogenate: 1 Garlic tissue homogenate: 1 Pear tissue homogenate: 1 Romaine lettuce tissue homogenate: 1 Grape tissue homogenate: 1 Yellow peach tissue homogenate: 1 Plum tissue homogenate: 1 Note: The diluent is extracting solution. For dilution of other sample types, perform a pretest to confirm the dilution factor.

8. The key points of the assay

Prepare the fresh reaction working solution before use.

9. Operating steps

1. Blank tube: Add 2 mL of extracting solution into a 10 mL glass test tube. Standard tube: Add 2 mL of 10 μg/mL standard solution into a 10 mL glass test tube. Sample tube: Add 2 mL of sample into a 10 mL glass test tube. Control tube: Add 2 mL of sample into a 10 mL glass test tube.
2. Add 2 mL of acetic acid into each tube. Add 2 mL of reaction working solution into blank, standard, and sample tubes. Add 2 mL of control working solution into the control tube. Mix thoroughly with a vortex mixer.
3. Cover the mouth of the tube with plastic film and prick a small hole with a needle. Incubate in a 100°C water bath for 30 min, then cool with running water.
4. Mix thoroughly and transfer 0.2 mL of supernatant to the microplate. Measure the OD value at 520 nm with a microplate reader.

10. Calculation

For the sample:

Pro content (μg/g wet weight) = (ΔA_1 / ΔA_2) × c × V ÷ m × f

[Note]

ΔA_1: OD_{Sample} - $OD_{Control}$

ΔA_2: $OD_{Standard}$ - OD_{Blank}

c: Concentration of standard, 10 μg/mL

V: The volume of extracting solution for sample preparation, mL

m: The weight of sample, g

f: Dilution factor of sample before test

11. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three pear tissue 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 (µg/mL)	3.50	12.60	30.20
%CV	5.5	5.2	3.7

Inter-assay Precision

Three pear tissue 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 (µg/mL)	3.50	12.60	30.20
%CV	10.0	4.7	7.5

Recovery

Three samples of high, middle, and low concentrations were tested in parallel 6 times each to obtain an average recovery rate of 100%.

Recovery

	Standard 1	Standard 2	Standard 3
Expected Conc. (µg/mL)	8.5	18.5	28

	Standard 1	Standard 2	Standard 3
Observed Conc. (µg/mL)	8.8	17.0	29.1
Recovery rate (%)	104	92	104

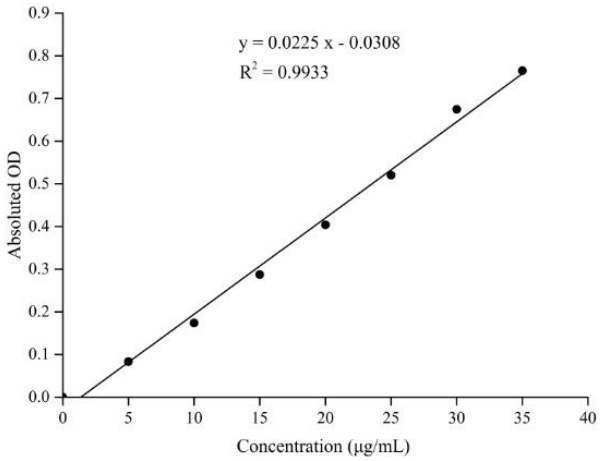
Sensitivity

The analytical sensitivity of the assay is 0.57 µg/mL. 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.

Standard curve

It is not necessary to prepare the standard curve for this kit; the provided standard curve is for reference only.

As the OD value of the standard curve may vary according to actual assay performance conditions (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only.



Standard curve

Concentration (µg/mL)	Average OD	Absoluted OD
0	0.042	0.000
5	0.125	0.084
10	0.216	0.174

Concentration (μg/mL)	Average OD	Absoluted OD
15	0.329	0.288
20	0.446	0.404
25	0.562	0.520
30	0.716	0.650
35	0.807	0.765

12. Appendix II Example Analysis

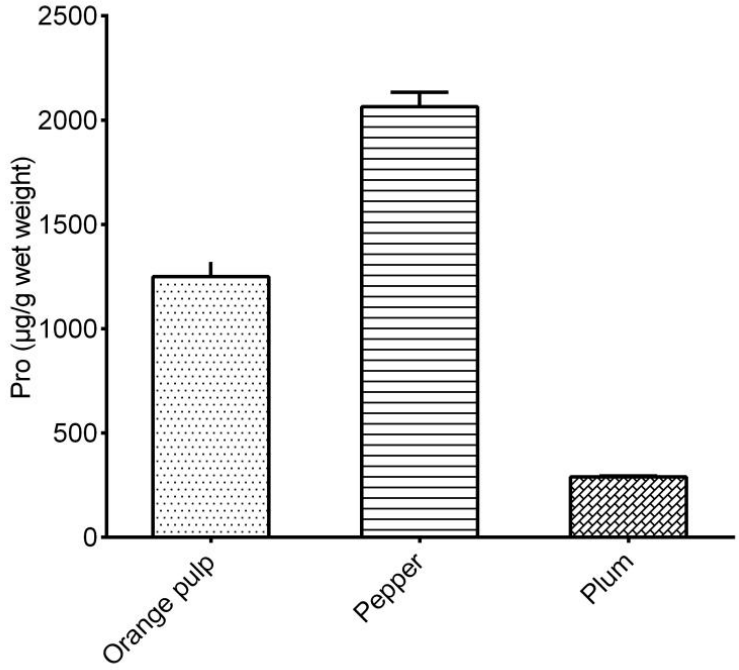
Example analysis:

Weigh 0.15 g of plum and cut into pieces, add 1.5 mL of extracting solution, homogenize the sample, centrifuge at 10,000 × g for 15 min at 4℃, then take 2 mL of sample and carry out the assay according to the operating steps. The results are as follows:

The average OD value of the sample is 1.555, the average OD value of the blank is 0.042, the average OD value of the standard is 0.188, the average OD value of the control is 1.119, and the calculation result is:

Pro content (μg/g wet weight) = (1.555 - 1.119) / (0.188 - 0.042) × 10 × 1.5 ÷ 0.15 = 298.6 μg/g wet weight

Orange pulp, pepper, and plum were detected according to the protocol; the results are as follows:



13. 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. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

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

6. The experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

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.