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

Catalog No：E-BC-K177-M

Specification：48T(22 samples)/96T(46 samples)

Measuring instrument：Microplate reader（510-530 nm）

Detection range：0.57-35 μg/mL

Elabscience® Proline (Pro) Colorimetric 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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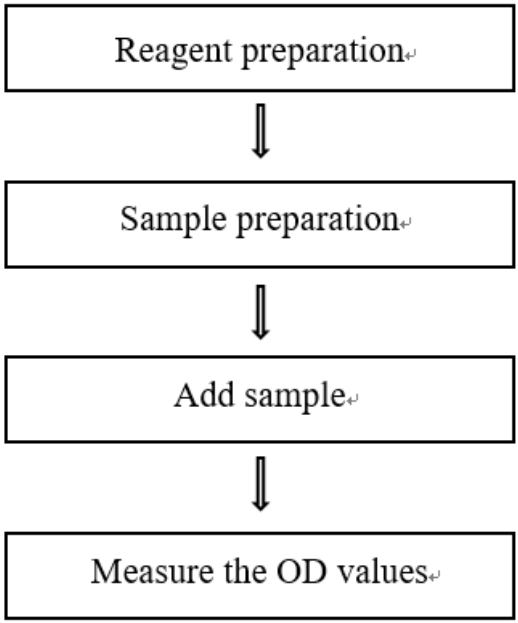
Assay summary

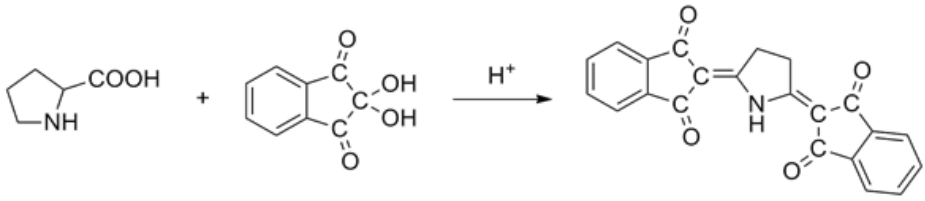


Intended use

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

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	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Extracting Solution	50 mL × 3 vials	60 mL × 5 vials	2-8 ℃, 12 months, shading light
Reagent 2	Ninhydrin	3 g × 1 vial	6 g × 1 vial	2-8 ℃, 12 months, shading light
Reagent 3	Acid Reagent	50 mL × 1 vial	50 mL × 2 vials	2-8 ℃, 12 months
Reagent 4	100 μg/mL Proline Standard	1 mL × 1 vial	2 mL × 1 vial	2-8 ℃, 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 (510-530 nm, optimum wavelength: 520 nm), Vortex mixer, Water bath, Centrifuge.

Reagents:

Double distilled water, Acetic acid

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of reaction working solution：

For each well, prepare 2 mL of reaction working solution (mix well 50 mg of ninhydrin, 1200 μL of acetic acid and 800 μL of acid reagent). Heat with agitation until it is dissolved completely (<70 ℃). Store the solution protected from light after cooling. The reaction working solution should be prepared on spot.

③ The preparation of control working solution：

For each well, prepare 2 mL of control working solution (mix well 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 on spot.

④ The preparation of 10 μg/mL standard solution：

For each well, prepare 2 mL of 10 μg/mL standard solution (mix well 200 μL of 100 μg/mL proline standard and 1800 μL of extracting solution). The 10 μg/mL standard solution should be prepared on spot.

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 ℃ water bath for 15 min (shake the tube constantly).

④ Then cool to room temperature, centrifuge at 10000 ×g for 15 min at 4 ℃, 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 ℃.

④ Centrifuge at 10000 ×g for 15 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

② Dilution of sample

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

Sample type	Dilution factor
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 the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

Prepare the fresh reaction working solution before use.

Operating steps

① Blank tube: Take 2 mL of extracting solution into the 10 mL glass test tube.
Standard tube: Take 2 mL of 10 μg/mL standard solution into the 10 mL glass test tube.
Sample tube: Take 2 mL of sample into the 10 mL glass test tube.
Control tube: Take 2 mL of sample into the 10 mL glass test tube.

② Add 2 mL of acetic acid into each tube. Add 2 mL of reaction working solution into blank tube, standard tube and sample tube. Add 2 mL of control working solution into control tube. 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 ℃ water bath for 30 min, then cool with running water.

④ Mix fully and take 0.2 mL of supernatant to the microplate and measure the OD value at 520 nm with microplate reader.

Calculation

The sample:

$$\text{Pro content (μg/g wet weight)} = \frac{\Delta A_1}{\Delta A_2} \times c \times V \div m \times f$$

[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.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three pear tissue 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 (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.

Parameters	Sample 1	Sample 2	Sample 3
Mean (μg/mL)	3.50	12.60	30.20
%CV	10.0	4.7	7.5

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 100%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (μg/mL)	8.5	18.5	28
Observed Conc. (μg/mL)	8.8	17.0	29.1
Recovery rate (%)	104	92	104

Sensitivity

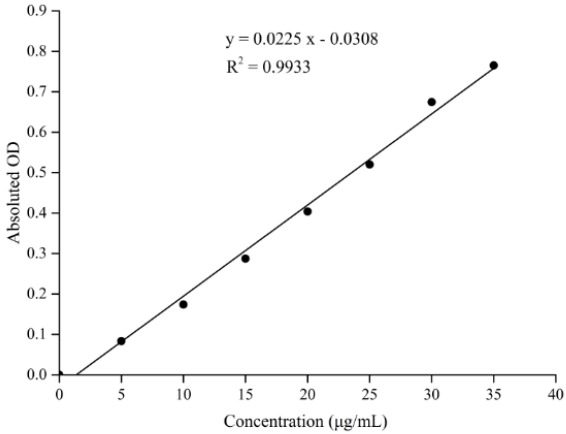
The analytical sensitivity of the assay is 0.57 μg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve

(It doesn't need to prepare the standard curve for this kit and the provided standard curve is for reference only)

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

Concentration (μg/mL)	0	5	10	15	20	25	30	35
Average OD	0.042	0.125	0.216	0.329	0.446	0.562	0.716	0.807
Absoluted OD	0.000	0.084	0.174	0.288	0.404	0.520	0.650	0.765



Appendix II Example Analysis

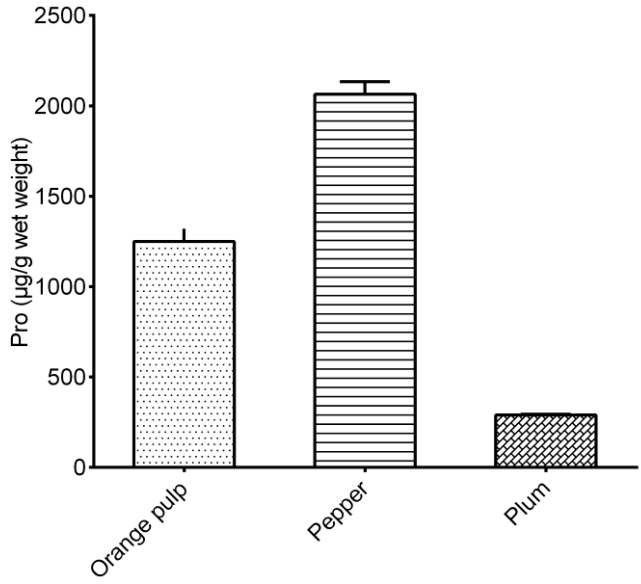
Example analysis:

Weight 0.15 g of plum and cut into pieces, add 1.5 mL of extracting solution, homogenized the sample, centrifuge at 10000×g for 15 min at 4℃, then take 2 mL of sample and carry the assay according to the operation 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:

$$\text{Pro content (μg/g wet weight)} = \frac{1.555\text{-}1.119}{0.188\text{-}0.042} \times 10 \times 1.5 \div 0.15 = 298.6 \text{ μg/g wet weight}$$

Detect orange pulp, pepper, plum 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.