Catalog No：E-BC-K177-S

Specification：50 Assays(48 samples)/ 100 Assays(96 samples)

Measuring instrument：Spectrophotometer（520 nm）

Detection range：0.17-35 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
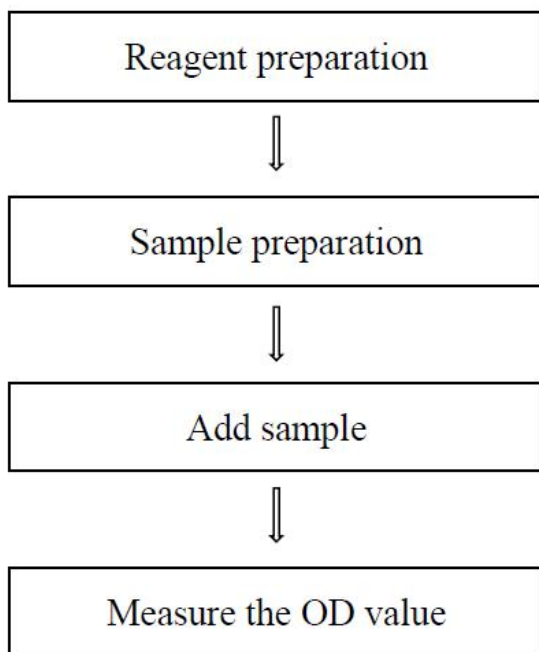Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

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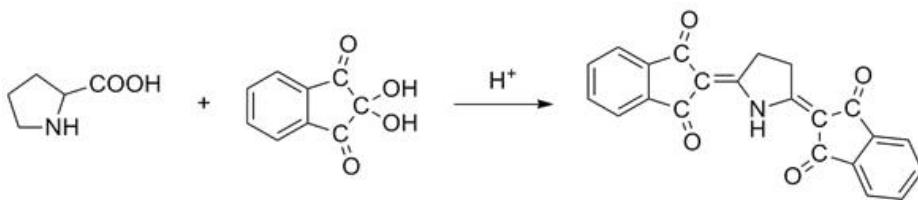
Intended use

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 (50 Assays)	Size 2 (100 Assays)	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

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:

Spectrophotometer (520 nm), Vortex mixer, Water bath, Centrifuge.

Reagents:

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

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of chromogenic agent：

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

③ 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

Honey sample:

① Harvest the amount of honey needed for each assay (initial recommendation 300 mg).

② Mix 300 mg honey in 3 mL extracting solution.

③ Incubate in 100°C water bath for 15 min (shake the tube constantly).

④ Then cool to room temperature, centrifuge at 10000×g for 15 min at 4°C, collect the supernatant for detection.

Tissue samples:

① Harvest the amount of tissue needed for each assay (initial recommendation 300 mg).

② Wash tissue in cold PBS (0.01 M, pH 7.4).

③ Homogenize 300 mg tissue in 3 mL extracting solution with a dounce homogenizer at 4°C.

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 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 extracting solution. For the dilution of other sample types, please do pretest to confirm the dilution factor.

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 H_2O and methylbenzene are not mutually soluble, it will affect the result.

③ The chromogenic agent must be fresh prepared.

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.

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.

⑤ 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 H_2O and methylbenzene are not mutually soluble, it will affect the result.)

Calculation

The sample:

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

[Note]

ΔA_1: $OD_{Sample} - OD_{Blank}$.

ΔA_2: $OD_{Standard} - OD_{Blank}$.

c: Concentration of standard, 10 μg/mL.

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

W: The weight of sample, g.

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three honey 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)	2.80	16.40	28.50
%CV	4.7	4.4	4.4

Inter-assay Precision

Three honey 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)	2.80	16.40	28.50
%CV	5.1	5.8	5.3

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 105%.

	Standard 1	Standard 2	Standard 3
Expected Conc. (µg/mL)	6.5	18	27.6
Observed Conc. (µg/mL)	6.9	18.5	29.3
Recovery rate (%)	106	103	106

Sensitivity

The analytical sensitivity of the assay is 0.17 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	30	35
Average OD	0.003	0.152	0.330	0.487	0.675	1.008	1.203
Absoluted OD	0	0.149	0.327	0.484	0.672	1.005	1.200

$y = 0.03432 x - 0.01567$
$R^2 = 0.99916$
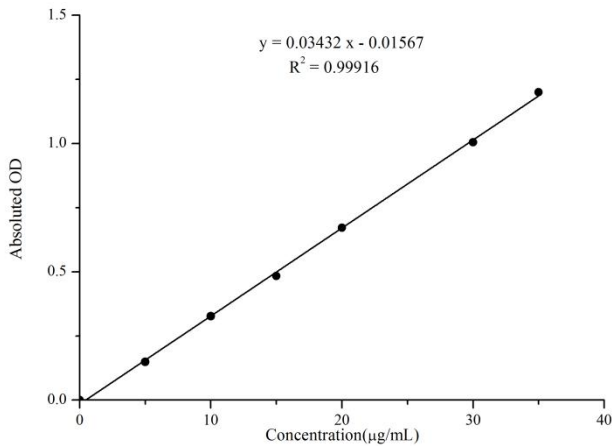
Appendix II Example Analysis

Example analysis：

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

$$\text{Pro content (μg/g)} = \frac{0.065 - 0.004}{0.444 - 0.004} \times 10 \text{ μg/mL} \times 5 \text{ mL} \div 0.5 \text{ g} \times 2.5 = 34.66 \text{ μg/g}$$

Detect green pepper, cabbages, daucus carota according to the protocol, the result is as follows：
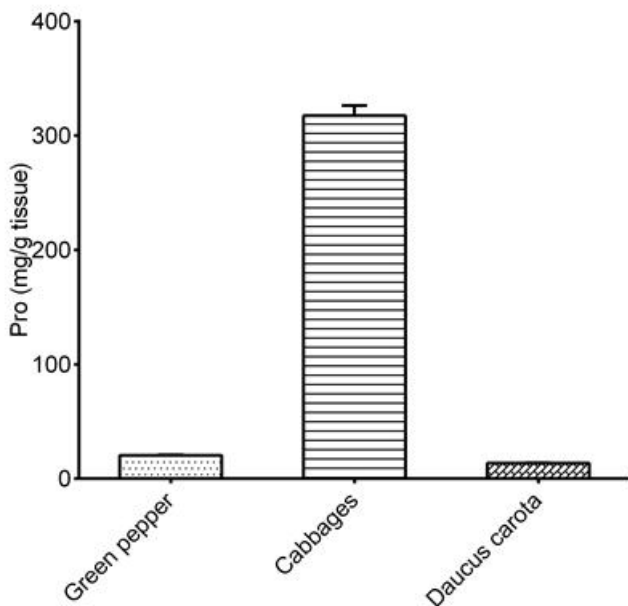
Statement

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.