

Proline (PRO) Colorimetric Assay Kit

Cat #: orb1173214 (manual)

Product Features

Product name: Proline (PRO) Colorimetric Assay Kit

Catalog number: orb1173214

Range: 0.5-80 µg/mL

Sensitivity: 0.5 µg/mL

Sample Types: Plant Tissues, Animal Tissues, Cells, Bacteria, Serum, Plasma

Storage: Stored at 4°C for 6 months, protected from light.

Assay Principle

Proline (PRO) is widely present in animals, plants, microorganisms and cultured cells. Under adversity conditions, the PRO content in plants increased significantly. The increased amount of PRO reflects stress resistance to a certain extent, and varieties with strong drought resistance tend to accumulate more PRO. Therefore, the increased amount of PRO can be used as one of the physiological indicators of stress resistance breeding. The kit can detect plant tissues, animal tissues, cells, bacteria, serum (plasma) and other samples. PRO is extracted with sulfosalicylic acid (SA). After heat treatment, Pro reacts with acidic ninhydrin solution to produce red; after extraction with toluene, the absorbance is measured at 520 nm.

Kit Components

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	60 mL	120 mL	4°C
Assay Buffer	7 mL	14 mL	4°C
Chromogen	7 mL	14 mL	4°C, protected from light
PRO Standard	Powder×1 vial (10 mg)	Powder×1 vial (10 mg)	4°C, protected from light

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 520 nm
- 96-well plate or microglass cuvette
- Ice Maker, centrifuge
- Precision pipettes, disposable pipette tips

- Deionized water, toluene
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C, protected from light.

PRO Standard: Before use, add 0.1 mL deionized water to the 1 mg PRO Standard powder and mix well to prepare 10 mg/mL Standard. Storage at 4°C, protected from light. The remaining working solution can be stored at 4°C, protected from light for 3 days. 10 mg powder should be weighed and dissolved in batches.

Setting of Standard Curves: Dilute 10 µL PRO Standard (10 mg/mL) to 100 µg/mL with 990 µL deionized water. And dilute the standard furtherly with deionized water as shown in the table below:

Num.	Volume of 100 µg/mL Standard (µL)	Volume of Deionized Water (µL)	Concentration (µg/mL)
Std.1	160	40	80
Std.2	80	120	40
Std.3	40	160	20
Std.4	20	180	10
Std.5	8	192	4
Std.6	4	196	2
Std.7	2	198	1
Std.8	1	199	0.5
Blank	0	200	0

Note: Always prepare a fresh set of standards per use. Diluted standard solution is unstable and must be used within 4 h.

Sample Preparation

1. Plant or Animal Tissue samples: Weigh 0.1 g samples and add 1 mL Extraction Buffer. Homogenize on ice. Take boiling water bath for 10 min. Centrifuge at 10,000 g for 10 min at room temperature, aspirating the supernatant, do further testing after cooling.
2. Bacteria or Cell sample: Collect bacteria or cells into a centrifuge tube. Then discard the supernatant after centrifugation. Add 1 mL Extraction Buffer for every 5×10^6 bacteria or cells. Ultrasonically break bacteria or cells (power 20%, work 3 s, intermittent 10 s, work 30 times). Take boiling water bath with shaking for 10 min. Centrifuge at 10,000 g for 10 min at room temperature, aspirating the supernatant, do further testing after cooling.

3. Serum or Plasma sample: Take 0.1 mL sample and add 0.9 mL Extraction Buffer. Mix well and take boiling water bath with shaking for 10 min. Centrifuge at 10,000 g for 10 min at room temperature, aspirating the supernatant, do further testing after cooling.

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for 1 month. It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat # orb1147876, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 520 nm, visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement

Reagent	Standard Tube (μL)	Blank Tube (μL)	Test Tube (μL)
Sample	0	0	100
Different Concentration Std.	100	0	0
Deionized Water	0	100	0
Assay Buffer	100	100	100
Chromogen	100	100	100
Add the reagents in sequence into the EP tube with a cap. Mix well and incubate in boiling water bath for 30 min (cover tightly to prevent water loss), shake once every 10 min. Cool to room temperature.			
Toluene	200	200	200
Shake for 30 s and let it stand for a while; add 100 μL supernatant into 96-well plate or microglass cuvette, measure the absorbance value at 520 nm with a microplate reader and record it as A. Calculate $\Delta A_{\text{Test}} = A_{\text{Test}} - A_{\text{Blank}}$, $\Delta A_{\text{Standard}} = A_{\text{Standard}} - A_{\text{Blank}}$.			

Note: To guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ΔA_{Test} values are higher than 1.5, dilute sample with Extraction Buffer and repeat this assay, multiply the results with the dilution factor. Increase the sample size if the ΔA_{Test} value is below 0.005. Toluene is toxic and is recommended in the fume hood.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the y-axis and the $\Delta A_{\text{Standard}}$ as the x-axis, draw the standard curve.

2. Calculating the content of PRO:

Bring the ΔA_{Test} into the equation to get the y value (μg/mL).

- (1) Calculated by protein concentration

$$\text{PRO } (\mu\text{g}/\text{mg prot}) = y \times V_{\text{Total}} \div (V_{\text{Sample}} \times C_{\text{pr}}) = \mathbf{3 \times y \div C_{\text{pr}}}$$

(2) Calculated by fresh weight of samples

$$\text{PRO } (\mu\text{g/g fresh weigh}) = y \times V_{\text{Total}} \div (V_{\text{Sample}} \div V_{\text{Sample Total}} \times W) = 3 \times y \div W$$

(3) Calculated by cell or bacteria numbers

$$\text{PRO } (\mu\text{g}/10^4) = y \times V_{\text{Total}} \div (500 \times V_{\text{Sample}} \div V_{\text{Sample Total}}) = 3 \times y \div 500$$

(4) Calculated by volumet of liquid samples

$$\text{PRO } (\mu\text{g/mL}) = y \times V_{\text{Total}} \div V_{\text{Sample}} = 3 \times y$$

Where:

V_{Total} : total reaction volume, 0.3 mL;

V_{Sample} : sample volume added, 100 μL =0.1 mL;

Cpr: sample protein concentration, mg/mL;

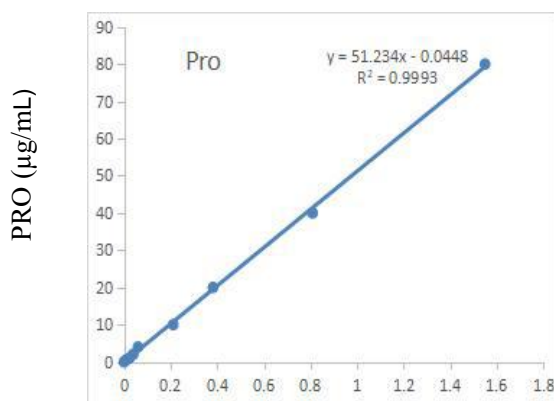
$V_{\text{Sample Total}}$: extract buffer added to samples, 1 mL;

W: fresh weigh of samples, g;

500: Total number of bacteria or cells, 5×10^6 .

Typical Data

Typical standard curve:



A520

Figure 1. Standard curve of PRO assay, data provided for demonstration purposes only. A new standard curve must be generated for each assay

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.