Proline Assay Kit

(Cat/No.:BC143 Size:50T/48S)

1. Principle of Measurement

Among the amino acids in plant, only proline can react with ninhydrin with stable red product give. The product has a maximum optical density (OD) at 515 nm. Thus the concentration of proline can be measured with acidic ninhydrin in presence. Other negative and neutral amino acids cannot react with acidic ninhydrin with red product. Positive amino acid interfere the reaction with negligible effect. Also, the addition of permutite may eliminate such interference

2. Compositions and Preparation (the kit is vaild for 3 months)

50T/ 48 Samples	Functions	Format	Preservation Condition
Reagent I	Homogenate Medium	2 Bottles×60 ml	4°C without light
Reagent II	Buffer Solution	1 Bottle×60 ml	4°C without light
Reagent III	Chromogenic Agent	1 Bottle×60 ml	4°C without light
Reagent IV	100μg/ ml Stock Solution	1 Bottle×1 ml	4°C
Preparation of Reagent IV Standard Solution (5μg/ ml): Extract 0.5 ml stock solution and dilute with reagent I to a final volume of 10 ml.			

3. Procedures

1. Pretreatment

Weigh the sample precisely and add reagent I with the ratio of 1 g tissue to 9 ml reagent I. Homogenize the mixture and centrifuge at 3,500 rpm for 10 min. Extract the supernatant.

2. Procedures

Compositions (ml)	Blank	Standard	Sample
Reagent I	0.5		
Reagent IV Solution		0.5	
Sample			0.5
Reagent II	1	1	1
Reagent III	1	1	1

Heat the tubes in the boiled water bath for 30 min. Cool the tube with water. Zero the 1 cm path length cuvettes with double distilled water (DDW) and record the OD values at 520 nm.

4. Calculation Formula

$$\underset{\mu g/g}{Proline\ Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Stadard} - OD_{Blank}} \times \frac{C_{Standard}}{5\mu g/ml} \times \frac{V_{Reagent\ I}}{m_{Tissue}} \times \underset{Pretreatment}{CoD}$$

Note: CoD represents the coefficient of dilution in the pretreatment process.

5. Example

Salt treated rye leaves along with normal rye was treated by 0.2 g rye leaves with 1.8 ml reagent I. The normal sample was measured directly with the supernatant obtained. The salt treated supernatant was diluted with reagent I to 10 times of its initial volume. The OD value for the normal sample was 0.514 and for treated one, the OD value was 0.471. The OD values for blank and standard tubes were 0.004 and 0.329 respectively.

Normal:

$$\underset{\mu g/g}{Proline\ Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Stadard} - OD_{Blank}} \times \frac{C_{Standard}}{5\mu g/ml} \times \frac{V_{Reagent\ I}}{m_{Tissue}} \times \underset{Pretreatment}{CoD}$$

$$= \frac{0.514 - 0.004}{0.329 - 0.004} \times 5 \times \frac{1.8}{0.2} \times 1 = 70.62\mu g/g$$

Slat treated

$$\underset{\mu g/g}{Proline\ Conc.} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Stadard} - OD_{Blank}} \times \frac{C_{Standard}}{5\mu g/ml} \times \frac{V_{Reagent\ I}}{m_{Tissue}} \times \underset{Pretreatment}{CoD}$$

$$= \frac{0.471 - 0.004}{0.329 - 0.004} \times 5 \times \frac{1.8}{0.2} \times 10 = 6.466 \times 10^2 \mu g/g$$

6. Notes

I. It is recommended to operate the measurement in the fume cupboard with mask and gloves.

II. Disposable capped plastic tubes are recommended for the measurement are available by the Institut

Appendix I: Proline Standard Curve Establishment

1. Pretreatment

Dilute the 100μg/ml reagent IV stock solution reagent I to the solutions with the concentrations mentioned below: 1, 2, 4, 5, 8, 10, 20μg/ ml.

Compositions (ml)	Blank	Standard
Reagent I	0.5	
Reagent IV with different Conc.		0.5
Reagent II	1	1
Reagent III	1	1

2. Procedure

Heat the tubes in the boiled water bath for 30 min. Cool the tube with water. Zero the 1 cm path length cuvettes with double distilled water (DDW) and record the OD values at 520 nm.

3. Results

Concentration (μg/ mg)	OD Measured	Absolute OD $\Delta OD = OD_{Measured} - OD_{Blank}$
0	0.004	0
1	0.065	0.061
2	0.128	0.124
4	0.258	0.254
5	0.329	0.325
8	0.528	0.524
10	0.673	0.669
20	1.345	1.341

4. Standard Curve