Proline (Pro) Content Assay Kit

Note: Take two or three different samples for prediction before test.

Detection equipment: Spectrophotometer/ Microplate reader

Cat No: BC0295

Size: 100T/96S

Product Composition: Before use, please carefully check whether the volume of the reagent is consistent with the volume in the bottle. If you have any questions, please contact Solarbio staff in time.

Reagent name	Size	Preservation Condition
Extract solution	Liquid 110 mL×1	2-8℃
Reagent I	Liquid 35 mL×1(Requird but not provided)	2-8℃
Reagent II	Liquid 35 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Reagent I: Glacial acetic acid 35mL, required but not provided. Storage at 2-8℃.
2. Standard: Proline 10 mg. Storage at 2-8℃. Dissolve in 1 mL of distilled water prepare as 10 mg/mL standard solution before use.

Description:

Proline (Pro) is widely found in animals, plants, microbe and culture cells. Under adverse condition, the content of Pro in plants increases significantly. The increase of Pro reflects the resistance in some extent, and the breeds with strong drought resistance tend to accumulate more proline. Therefore, the increase of proline can be used as one of the physiological indexes of stress resistance breeding.

After Pro is extracted by sulfosalicylic acid (SA), Pro reacted with acid ninhydrin solution to form something red. The absorbance of the red material is determined by 520 nm after extraction with toluene.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, water bath, desk centrifuge, transferpettor, micro glass cuvette/96 well flat-bottom plate, mortar/homogenizer, **glacial acetic acid (>98%, AR)**, ice and distilled water.

Protocol:

I. Sample preparation:

1. Cells or Bacteria: Collect bacteria or cells into the centrifuge tube. Suggest 5 million with 1 mL of Extract reagent. Use ultrasonication to splitting bacteria and cell (placed on ice, ultrasonic power 200W, working time 3s, interval 10s, repeat for 30 times). Incubate at boiling water for 10 minutes(Wrap the sealing film to prevent bursting). After cooling, centrifuge at 10000 ×g for 10 minutes at room temperature, take the supernatant for test.

2. Tissue: Add 1 mL of Extract solution to 0.1 g of tissue, and fully homogenized on ice.Incubate at boiling water for 10 minutes(Wrap the sealing film to prevent bursting). After cooling, centrifuge at 10000 ×g

for 10 minutes at room temperature, take the supernatant for test.

3. Serum: Add 0.9 mL of Extract solution to 100 μL of serum, mix thoroughly. Incubate at boiling water for 10 minutes(Wrap the sealing film to prevent bursting). After cooling, centrifuge at room temperature for 10 minutes, take the supernatant for test.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 520 nm, spectrophotometer set zero with distilled water.

2. Standard: Dilute the 10 mg/mL standard solution to 40, 20, 10, 8, 4, 2, 1, 0.5μg/mL standard with distilled water

3. Add reagents with the following list:

Reagent（mL）	Test tube (T)	Standard tube (S)	Black tube (B)
Sample	0.25	-	-
Standard	-	0.25	-
Distilled water	-	-	0.25
Reagent I	0.25	0.25	0.25
Reagent II	0.25	0.25	0.25
Incubate at boiling water for 30 minutes (cover tightly to prevent moisture loss). Vibrate for every 10 minutes. After cooling, add 0.2 mL of the mixture into micro glass cuvette/96 well plate, and detect the absorbance value of each tube at 520 nm. Record as A_T, A_S, A_B and calculate $\Delta A=A_T-A_B$， $\Delta A_S=A_S-A_B$. Blank tube only need to be measured once or twice.			

III. Calculation

1. According to the concentration of the standard tube (x, μg/mL) and the absorbance ΔAs (y, ΔAs), a standard curve was established. According to the standard curve, ΔA (y, ΔA) was brought into the formula to calculate the sample concentration (x, μg/mL).

2. Bacteria or cells

Pro content (μg/10^4 cell) = $x\times V_{ST}\div N = x \div N$

3. Tissue weight

Pro content (μg/g weight) = $x\times V_{ST}\div W=x\div W$

4. Serum(plasma) volume

Pro content (μg/mL)=10×x

V_{ST}: Extract solution volume, 1 mL;

N: Total number of bacteria and cells, count by 10^4;

W: Sample weight, g;

10: Serum dilution ratio, $(0.1+0.9)\div 0.1=10$.

Note:

1. Extract solution has protein precipitate, the supernatant can not be used for the detection of protein concentration.

2. If the absorbance value exceeds the linear range, the sample size can be increased or diluted before determination.

Recect Product Citations:

[1] Yanan Wang,Chengzhen Liang,Zhigang Meng,et al. Leveraging Atriplex hortensis choline monooxygenase to improve chilling tolerance in cotton. Environmental and Experimental Botany. June 2019;162:364-373.(IF3.712)

[2] Zeyong Zhang,Huanhuan Liu,Ce Sun,et al. A C2H2 zinc-finger protein OsZFP213 interacts with OsMAPK3 to enhance salt tolerance in rice. Journal of Plant Physiology. October 2018;(IF2.825)

[3] Huang Q, Wang M, Xia Z. The SULTR gene family in maize (Zea mays L.): gene cloning and expression analyses under sulfate starvation and abiotic stress[J]. Journal of plant physiology, 2018, 220: 24-33.

[4] Chen M X, Zhu F Y, Wang F Z, et al. Alternative splicing and translation play important roles in hypoxic germination in rice[J]. Journal of experimental botany, 2019, 70(3): 817-833.

[5] Zhao Y, Yu W, Hu X, et al. Physiological and transcriptomic analysis reveals the involvement of crucial factors in heat stress response of Rhododendron hainanense[J]. Gene, 2018, 660: 109-119.

References:

[1] Vieira S M, Silva T M, Glória M B A. Influence of processing on the levels of amines and proline and on the physico-chemical characteristics of concentrated orange juice[J]. Food chemistry, 2010, 119(1): 7-11.

[2] Demiral T, Türkan I. Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance[J]. Environmental and experimental botany, 2005, 53(3): 247-257.

Related Products:

BC1550/BC1555	Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit
BC1560/BC1565	Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit
BC0180/BC0185	Cysteine(Cys) Content Assay Kit
BC1580/BC1585	Glutamic Acid(Glu) Content Assay Kit

Technical Specifications:

The detection limit: 0.2876 μg/mL

Linear range: 0.5-30 μg/mL