Proline (PRO) Conrent Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: BC0290

Size: 50T/48S

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 60 mL×1	2-8℃
Reagent I	Liquid 45 mL×1(Requird but not provided)	2-8℃
Reagent II	Liquid 45 mL×1	2-8℃
Standard	Powder×1	2-8℃

Solution Preparation:

1. Reagent I: Ice acetic acid 45mL, required but not provided. Storage at 2-8℃.
2. Standard: Proline 10 mg. Storage at 2-8℃. Before use, 1 mL water iss added to prepare 10 mg/mL standard solution.

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 can determined by 520 nm after extraction with toluene.

Reagents and Equipment Required but Not Provided:

Spectrophotometer, water bath, desk centrifuge, transferpettor, 1 mL glass cuvette, glacial acetic acid (>98%, AR), methylbenzene, mortar/homogenizer, 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. 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. 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. After cooling, centrifuge at 10000 ×g for 10 minutes at room temperature, take the supernatant for test.

II. Determination procedure:

1. Preheat spectrophotometer for 30 minutes, adjust wavelength to 520 nm, set zero with methylbenzene .
2. Standard: Dilute the 10 mg/mL standard solution to 40, 20, 10, 8, 4, 2, 1, 0.5 μg/mL standard with distilled water.
3. Add reagents with the following list:

Reagent (mL)	Test tube(T)	Standard tube(S)	Blank tube(B)
Sample	0.5	-	-
Standard	-	0.5	-
Distilled water	-	-	0.5
Reagent I	0.5	0.5	0.5
Reagent II	0.5	0.5	0.5
After mixing, cover tightly, wrap the sealing film, and keep warm in the boiling water bath for 30min, oscillate every 10min, and then compare colors at 520nm wavelength after cooling, record as A_T, A_S and A_B, and calculate $\Delta A= A_T - A_B$, $\Delta A_S = A_S - A_B$. Blank tubes only need to be done 1-2 times.			

III. Calculation

1. According the standard curve to calculate sample proline (PRO) content. (x: proline content, μg/mL; y: absorbance value).
2. Calculate according to the number of bacteria, cells or fresh weight of tissues. First, calculate the x value according to the standard curve:

1) Bacteria or cells

Pro (μg/10^4 cell) = $x \times V_E \div N_C = x \div N_C$

2) Tissue weight

Pro (μg/g fresh weight) = $x \times V_E \div W = x \div W$(g/mL)

3) Serum (plasma)

Pro (μg/mL Serum (plasma))=10×x

V_E: Add the Extraction liquid volume, 1mL

Nc: Total number of cells or bacteria, 10^4 cell/mL

W: Sample weight, g

10: Serum dilution ratio, (0.1+0.9) ÷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] Zhang D, Liu J, Zhang Y, Wang H, Wei S, Zhang X, Zhang D, Ma H, Ding Q, Ma L. Morphophysiological, proteomic and metabolomic analyses reveal cadmium tolerance mechanism in common wheat (Triticum aestivum L.). J Hazard Mater. 2023 Mar 5;445:130499. doi: 10.1016/j.jhazmat.2022.130499. Epub 2022 Nov 25. PMID: 36455318.

[2] Liu X, Cheng C, Min Y, Xie X, Muzahid ANM, Lv H, Tian H, Zhang C, Ye C, Cao S, Chen P, Zhong C, Li D. Increased ascorbic acid synthesis by overexpression of AcGGP3 ameliorates copper toxicity in kiwifruit. J Hazard Mater. 2023 Oct 15;460:132393. doi: 10.1016/j.jhazmat.2023.132393. Epub 2023 Aug 23. PMID: 37660623.

[3] Liu K, Chen J, Sun S, Chen X, Zhao X, Hu Y, Qi G, Li X, Xu B, Miao J, Xue C, Zhou Y, Gong Z. Histone deacetylase OsHDA706 increases salt tolerance via H4K5/K8 deacetylation of OsPP2C49 in rice. J Integr Plant Biol. 2023 Jun;65(6):1394-1407. doi: 10.1111/jipb.13470. Epub 2023 Apr 19. PMID: 36807738.

[4] Qiao Z, Sun X, Gong K, Zhan X, Luo K, Fu M, Zhou S, Han Y, He Y, Peng C, Zhang W. Toxicity of decabromodiphenyl ethane on lettuce: Evaluation through growth, oxidative defense, microstructure, and metabolism. Environ Pollut. 2023 Dec 1;338:122724. doi: 10.1016/j.envpol.2023.122724. Epub 2023 Oct 11. PMID: 37832780.

[5] Xu T, Wu Z, Yuan Q, Zhang X, Liu Y, Wu C, Song M, Wu J, Jiang J, Wang Z, Chen Z, Zhang M, Huang M, Ji N. Proline is increased in allergic asthma and promotes airway remodeling. JCI Insight. 2023 Aug 22;8(16):e167395. doi: 10.1172/jci.insight.167395. PMID: 37432745; PMCID: PMC10543727.

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: 0.1791 μg/mL

Linear range: 0.5-40 μg/mL