SunLong Biotech Co.,LTD
Tel: 0086-571-56623320 Fax:0086-571-56623318
E-mail:sales@sunlongbiotech.com
www.sunlongbiotech.com

Proline (PRO) Content Assay Kit

Note: Take two or three different samples for prediction before test.
Detection equipment: Spectrophotometer/ Microplate reader

Cat No: AK0563

Size: 100T/96S

Components:
Extract solution: Liquid 100 mL×1. Storage at 4℃ .

Reagent I: Ice acetic acid, required but not provided. Storage at 4℃ .

Reagent II: Liquid 35 mL×1. Storage at 4℃ .

Reagent III: Methylbenzene, required but not provided. Storage at 4℃ .

Standard: Powder×1, proline 10 mg. Storage at 4℃ . Dissolve in 1 mL of distilled water prepare as 10 mg/mL standard solution before use.

Description:
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, ice acetic acid, methylbenzene, 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 20%, 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 for 10 minutes, take the supernatant for test.

4. Standard: Dilute the 10 mg/mL standard solution to 15, 10, 8, 6, 4, 2, 1 μg/mL standard with distilled water.

II. Determination procedure:

1. Preheat spectrophotometer/microplate reader for 30 minutes, adjust wavelength to 520 nm, set zero with methylbenzene .
2. Take 0.25 mL of supernatant(or diluted standard), 0.25 mL of Reagent I and 0.25 mL of Reagent II to the 2 mL of centrifuge tube. Incubate at boiling water for 30 minutes (cover tightly to prevent moisture loss). Vibrate for every 10 minutes.
3. After cooling, add 0.5 mL of Reagent III, vibrate for 30 seconds, place a few minutes, transfer pigment into Reagent III, take 0.2 mL of the upper liquid to micro quartz cuvette. Record the absorbance at 520 nm wavelength.
4. Establish the proline standard curve according to the absorbance and concentration.

III. Calculation

1. According the standard curve to calculate sample proline (PRO) content. (y: proline content, μg/mL; x: absorbance value).
2. Bacteria or cells

PRO (μg/10^4 cell) = $y \times V_{ST} \div N_C = y \div N_C$

3. Tissue weight

PRO (μg/g fresh weight) = $y \times V_{ST} \div W = y \div W$

4. Serum(plasma)

PRO (μg/mL)=$10 \times y$

V_{ST} : Extract solution volume, 1 mL;
N_C : Total number of bacteria and cells, 10^4 is a unit;
W: Sample weight, g;
10: Serum dilution ratio, (0. 1+0.9)÷0. 1=10.

Note:

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

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 revealed 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:

AK0423/AK0422 Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit
AK0421/AK0420 Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit
AK0582/AK0581 Cysteine(Cys) Content Assay Kit
AK0417/AK0416 Glutamic Acid(Glu) Content Assay Kit

Technical Specifications:

The detection limit: 0.2876 μg/mL

Linear range: 0.5-30 μg/mL