

Proline (PRO) Content Assay Kit

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

Detection equipment: Spectrophotometer

Cat No: NA0845

Size: 50T/48S

Components:

Extract solution: Liquid 50 mL×1. Storage at 4°C.

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

Reagent II: Liquid 45 mL×1. Storage at 4°C.

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

Standard: Powder×1, proline 10 mg. Storage at 4°C.

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 be 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, mortar, ice acetic acid, 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 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, 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 20, 10, 8, 6, 4, 2, 1, 0 $\mu\text{g/mL}$ standard with distilled water.
3. Take 0.5 mL of supernatant (or diluted standard), 0.5 mL of Reagent I and 0.5 mL of Reagent II to the tube. Incubate at boiling water for 30 minutes (cover tightly to prevent moisture loss). Vibrate for every 10 minutes.
4. After cooling, add 1 mL of Reagent III to the tube, vibrate for 30 seconds, place a few minutes, make pigment transfer into Reagent III. Take 0.8mL- 1mL of the upper liquid to 1 mL glass cuvette. Record the absorbance at 520 nm wavelength.
5. 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, $\mu\text{g/mL}$; x: absorbance value).
2. Calculate according to the number of bacteria, cells or fresh weight of tissues. First, calculate the y value according to the standard curve:

1) Bacteria or cells

$$\text{PRO } (\mu\text{g}/10^4 \text{ cell}) = y \div N_c(10^4 \text{ cell/mL})$$

2) Tissue weight

$$\text{PRO } (\mu\text{g/g fresh weight}) = y \div W(\text{g/mL})$$

3. Serum (plasma)

$$\text{PRO } (\mu\text{g/mL Serum (plasma)}) = y \div 0.1 \text{ mL(Serum (plasma)/mL)}$$

N_c : Total number of cells or bacteria;

W: Sample weight, g.

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.

Recent 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:

NA0744/NA0502 Glutamic-pyruvic Transaminase(GPT) Activity Assay Kit

NA0743/NA0501 Glutamic-oxalacetic Transaminase(GOT) Activity Assay Kit

NA0856/NA0614 Cysteine(Cys) Content Assay Kit

NA0741/NA0499 Glutamic Acid(Glu) Content Assay Kit

Technical Specifications:

The detection limit: 0.1791 µg/mL

Linear range: 0.25-20 µg/mL