CheKine™ Micro Proline (PRO) Assay Kit

Cat #: KTB1430 Size: 48 T/96 T

	Micro Proline (PRO) Assay Kit		
REF	**Cat #:** KTB1430	LOT	**Lot #:** Refer to product label
	Detection range: 0.5-80 μg/mL		**Sensitivity:** 0.5 μg/mL
	Applicable samples: Plant Tissues, Animal Tissues, Cells, Bacteria, Serum, Plasma		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

Proline (PRO) is widely present in animals, plants, microorganisms and cultured cells. Under adversity conditions, the PRO content in plants increased significantly. The increased amount of PRO reflects stress resistance to a certain extent, and varieties with strong drought resistance tend to accumulate more PRO. Therefore, the increased amount of PRO can be used as one of the physiological indicators of stress resistance breeding. The kit can detect plant tissues, animal tissues, cells, bacteria, serum (plasma) and other samples. PRO is extracted with sulfosalicylic acid (SA). After heat treatment, Pro reacts with acidic ninhydrin solution to produce red; after extraction with toluene, the absorbance is measured at 520 nm.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	50 mL	100 mL	4℃
Assay Buffer	5 mL	10 mL	4℃
Chromogen	5 mL	10 mL	4℃, protected from light
PRO Standard	10 mg	10 mg	4℃, protected from light

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 520 nm
- 96-well plate or microglass cuvette
- Ice Maker, centrifuge
- Precision pipettes, disposable pipette tips
- Deionized water
- Toluene

• Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Assay Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃.

Chromogen: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4℃, protected from light.

PRO Standard: Before use, add 1 mL deionized water to the 10 mg PRO Standard powder and mix well to prepare 10 mg/mL Standard. Storage at 4℃, protected from light. The remaining working solution can be stored at 4℃ , protected from light for 3 days.

Setting of Standard Curves: Dilute 10 μL PRO Standard (10 mg/mL) to 100 μg/mL with 990 μL deionized water. And dilute the standard furtherly with deionized water as shown in the table below:

Num.	Volume of 100 μg/mL Standard (μL)	Volume of Deionized Water (μL)	Concentration (μg/mL)
Std.1	160	40	80
Std.2	80	120	40
Std.3	40	160	20
Std.4	20	180	10
Std.5	8	192	4
Std.6	4	196	2
Std.7	2	198	1
Std.8	1	199	0.5
Blank	0	200	0

Sample Preparation

1. Plant or Animal Tissue samples: Weigh 0.1 g samples and add 1 mL Extraction Buffer. Homogenize on ice. Take boiling water bath for 10 min. Centrifuge at 10,000 g for 10 min at room temperature, aspirating the supernatant, do further testing after cooling.
2. Bacteria or Cell sample: Collect bacteria or cells into a centrifuge tube. Then discard the supernatant after centrifugation. Add 1 mL Extraction Buffer for every 5×10^6 bacteria or cells. Ultrasonically break bacteria or cells (power 20%, work 3 s, intermittent 10 s, work 30 times). Take boiling water bath with shaking for 10 min. Centrifuge at 10,000 g for 10 min at room temperature, aspirating the supernatant, do further testing after cooling.
3. Serum or Plasma sample: Take 0.1 mL sample and add 0.9 mL Extraction Buffer. Mix well and take boiling water bath with shaking for 10 min. Centrifuge at 10,000 g for 10 min at room temperature, aspirating the supernatant, do further testing after cooling.

Note: It will be better to quantify the total protein with Protein Quantification Kit (BCA Assay), Cat #: KTD3001, if the content is calculated by protein concentration.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 520 nm, visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement:

Reagent	Standard Tube (μL)	Blank Tube (μL)	Test Tube (μL)
Sample	0	0	100

Different Concentration Std.	100	0	0
Deionized Water	0	100	0
Assay Buffer	100	100	100
Chromogen	100	100	100
Add the reagents in sequence into the EP tube with a cap. Mix well and incubate in boiling water bath for 30 min (cover tightly to prevent water loss), shake once every 10 min. Cool to room temperature.			
Toluene	200	200	200
Shake for 30 s and let it stand for a while; add 100 μL supernatant into 96-well plate or microglass cuvette, measure the absorbance value at 520 nm with a microplate reader and record it as A. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$, $\Delta A_{Standard}=A_{Standard}-A_{Blank}$.			

Note: In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the ΔA_{Test} values are higher than 1.5, dilute sample with Extraction Buffer and repeat this assay, multiply the results with the dilution factor.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Drawing of standard curve:

With the concentration of the standard solution as the y-axis and the $\Delta A_{Standard}$ as the x-axis, draw the standard curve.

2. Calculating the content of PRO:

Bring the ΔA_{Test} into the equation to get the y value (μg/mL).

(1) Calculated by protein concentration

PRO (μg/mg prot)=$y\times V_{Total}\div(V_{Sample}\times Cpr)$**=3×y÷Cpr**

(2) Calculated by fresh weight of samples

PRO (μg/g fresh weigh)=$y\times V_{Total}\div(V_{Sample}\div V_{Sample\ Total}\times W)$**=3×y÷W**

(3) Calculated by cell or bacteria numbers

PRO (μg/10^4)=$y\times V_{Total}\div(500\times V_{Sample}\div V_{Sample\ Total})$**=3×y÷500**

(4) Calculated by volumet of liquid samples

PRO (μg/mL)=$y\times V_{Total}\div V_{Sample}$**=3×y**

Where: V_{Total}: total reaction volume, 0.3 mL; V_{Sample}: sample volume added, 100 μL=0.1 mL; Cpr: sample protein concentration, mg/mL; $V_{Sample\ Total}$: extract buffer added to samples, 1 mL; W: fresh weigh of samples, g; 500: Total number of bacteria or cells, 5×10^6.

Typical Data

Typical standard curve:

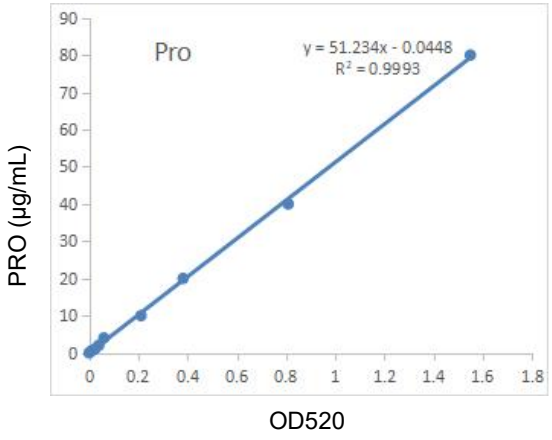

Figure 1. Standard curve of PRO assay, data provided for demonstration purposes only. A new standard curve must be generated for each assay

Recommended Products

Catalog No.	Product Name
KTB1410	CheKine™ Micro Alanine Aminotransferase (ALT/GPT) Activity Assay Kit
KTB1420	CheKine™ Micro Aspartate Aminotransferase (AST/GOT) Activity Assay Kit
KTB1440	CheKine™ Micro Glutamate (Glu) Assay Kit
KTB1450	CheKine™ Micro Cysteine (Cys) Assay Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.