



Proline Microplate Assay Kit

Catalog # AS0117

Detection and Quantification of Proline Content in Urine, Serum, Plasma, Tissue extracts, Cell lysate, Cell culture and Other biological fluids Samples.

This instruction must be read in its entirety before using this product.

For research use only, Not for use in diagnostic procedures.

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I. INTRODUCTION.....	2
II. KIT COMPONENTS.....	3
III. MATERIALS REQUIRED BUT NOT PROVIDED.....	3
VI. SAMPLE PREPARATION.....	4
V. ASSAY PROCEDURE.....	5
VI. CALCULATION.....	6
VII. TYPICAL DATA.....	7
VIII. TECHNICAL SUPPORT.....	7
IX. NOTES.....	7

I. INTRODUCTION

Proline (abbreviated as Pro or P; encoded by the codons CCU, CCC, CCA, and CCG) is an α -amino acid that is used in the biosynthesis of proteins. It contains an α -amino group (which is in the protonated $>\text{NH}_2^+$ form under biological conditions), an α -carboxylic acid group (which is in the deprotonated $-\text{COO}^-$ form under biological conditions), and a side chain pyrrolidine, classifying it as a nonpolar (at physiological pH), aliphatic amino acid. It is non-essential in humans, meaning the body can synthesize it from the non-essential amino acid L-glutamate.

Proline Microplate Assay Kit is a sensitive assay for determining Proline in various samples. Proline concentration is determined by Ninhydrin. The reaction products can be measured at a colorimetric readout at 520 nm.

II. KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30 ml x 4	4 °C
Reaction Buffer	5 ml x 1	4 °C
Dye Reagent	Powder x 1	4 °C
Dye Reagent Diluent	5 ml x 1	4 °C
Standard	Powder x 1	4 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Dye Reagent: Add 5 ml Dye Reagent Diluent into Dye Reagent bottle, heat to dissolve.

Standard: Add 1 ml distilled water to dissolve before use, then add 0.1 ml into 0.9 ml distilled water, the concentration will be 200 µg/ml.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 520 nm
2. Distilled water
3. Pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Convection oven

IV. SAMPLE PREPARATION

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5×10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, interval 10s, repeat 30 times); centrifuged at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer on ice; centrifuged at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples

Add 0.1 ml serum or plasma and 0.9 ml Assay buffer into the microcentrifuge tube, mix; centrifuged at 10000g 4°C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

V. ASSAY PROCEDURE

Add following reagents into the microplate:

Reagent	Sample	Standard	Blank
Sample	50 μ l	--	--
Standard	--	50 μ l	--
Distilled water	--	--	50 μ l
Reaction Buffer	50 μ l	50 μ l	50 μ l
Dye Reagent	50 μ l	50 μ l	50 μ l
Mix, put it into the convection oven, 90 °C for 20 minutes, mix, record absorbance measured at 520 nm.			

VI. CALCULATION

1. According to the protein concentration of sample

$$\begin{aligned} \text{Pro } (\mu\text{g}/\text{mg}) &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \\ &\quad (\text{V}_{\text{Sample}} \times \text{C}_{\text{Protein}}) \\ &= 200 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{C}_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{Pro } (\mu\text{g}/\text{g}) &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (\text{W} \times \text{V}_{\text{Sample}} / \\ &\quad \text{V}_{\text{Total}}) \\ &= 200 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{W} \end{aligned}$$

3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{Pro } (\mu\text{g}/10^4) &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (\text{V}_{\text{Sample}} \times \text{N} / \\ &\quad \text{V}_{\text{Assay}}) \\ &= 200 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{N} \end{aligned}$$

4. According to the volume of serum or plasma

$$\begin{aligned} \text{Pro } (\mu\text{g}/\text{ml}) &= (\text{C}_{\text{Standard}} \times \text{V}_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (\text{V}_{\text{Sample}} \times \text{V} / \\ &\quad \text{V}_{\text{Assay}}) \\ &= 200 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / \text{V} \end{aligned}$$

$\text{C}_{\text{Standard}}$: the standard concentration, 200 $\mu\text{g}/\text{ml}$;

$\text{C}_{\text{Protein}}$: the protein concentration, mg/ml;

W: the weight of sample, g;

V: the volume of serum or plasma, ml;

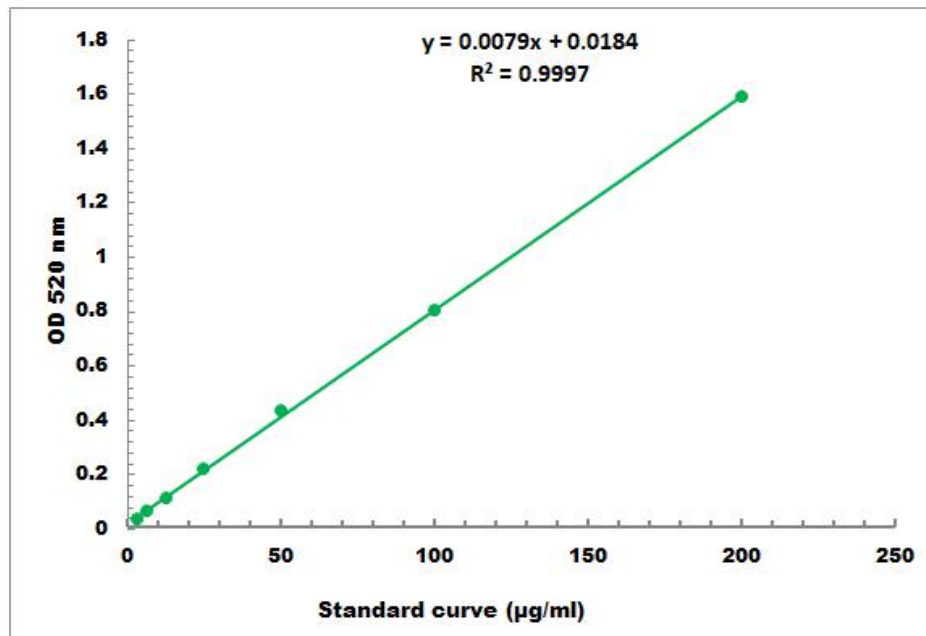
$\text{V}_{\text{Standard}}$: the volume of standard, 0.05 ml;

V_{Sample} : the volume of sample, 0.05 ml;

V_{Assay} : the volume of Assay buffer, 1 ml.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 2µg/ml - 200 µg/ml

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.sabbiotech.cn or contact us at techcn@signalwayantibody.com

IX. NOTES