



Proline Microplate Assay Kit

User Manual

Catalog # ASK1121

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

For research use only. Not for diagnostic or therapeutic procedures.

Bioworld Technology, Inc. (USA)

Email: info@bioworld.com

Web: www.bioworld.com

Bioworld technology, co. Ltd. (China)

Email: info@biogot.com

Web: www.biogot.com



I. INTRODUCTION.....2

II. KIT COMPONENTS.....3

III. MATERIALS REQUIRED BUT NOT PROVIDED.....3

IV. SAMPLE PREPARATION.....4

V. ASSAY PROCEDURE.....5

VI. CALCULATION.....6

VII. TYPICAL DATA.....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 $>NH_2^+$ form under biological conditions), an α -carboxylic acid group (which is in the deprotonated $-COO^-$ 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 µl	--	--
Standard	--	50 µl	--
Distilled water	--	--	50 µl
Reaction Buffer	50 µl	50 µl	50 µl
Dye Reagent	50 µl	50 µl	50 µl
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

$$\begin{aligned} \text{Pro } (\mu\text{g}/\text{mg}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times C_{\text{Protein}}) \\ &= 10 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

2. According to the weight of sample

$$\begin{aligned} \text{Pro } (\mu\text{g}/\text{g}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (W \times \\ &\quad V_{\text{Sample}} / V_{\text{Total}}) \\ &= 10 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

3. According to the quantity of cells or bacteria

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

4. According to the volume of serum or plasma

$$\begin{aligned} \text{Pro } (\mu\text{g}/\text{ml}) &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times V / V_{\text{Assay}}) \\ &= 10 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Blank}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V \end{aligned}$$

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

C_{Protein} : the protein concentration, mg/ml ;

W : the weight of sample, g ;

V : the volume of serum or plasma, ml ;

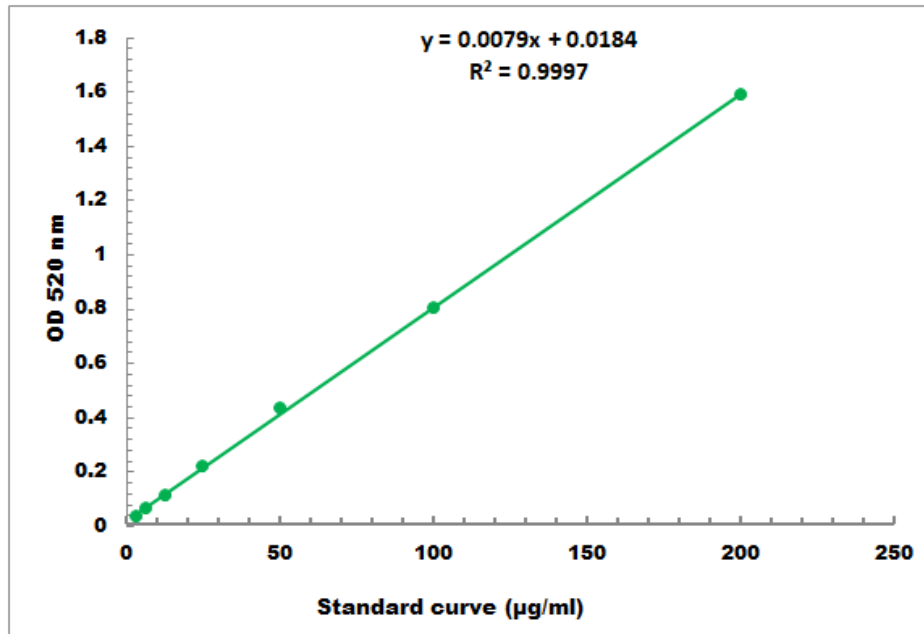
V_{Standard} : the volume of standard, 0.05 ml ;

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: 2 µg/ml - 200 µg/ml