Proline Microplate Assay Kit

User Manual

Catalog # CAK1121

(Version 1.2A)

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.

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 >NH2+ 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	
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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, multi-channel 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%, sonicate 3s, interval 10s, repeat 30 times); put it into boiling water bath for 10 minutes; centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube for detection.

2. For tissue samples

Weigh out 0.1 g tissue, homogenize with 1 ml Assay buffer; then transfer into centrifuge tube, put it into boiling water bath for 10 minutes; centrifuged at 10000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube 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; put it into boiling water bath for 10 minutes; centrifuged at 10000g for 10 minutes, take the supernatant into a new centrifuge tube 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.			

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

1. According to the protein concentration of sample

$$Pro\ (\mu g/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times C_{Protein})$$

$$= 200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$Pro\ (\mu g/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (W \times V_{Sample} / V_{Total})$$

$$= 200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / W$$

3. According to the quantity of cells or bacteria

$$Pro\ (\mu g/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay})$$

$$= 200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of serum or plasma

$$Pro\ (\mu g/ml) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times V / V_{Assay})$$

$$= 200 \times (OD_{Sample} - OD_{Blank}) / (OD_{Standard} - OD_{Blank}) / V$$

$C_{Standard}$: the standard concentration, 200 μg/ml;

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

W: the weight of sample, g;

V: the volume of serum or plasma, ml;

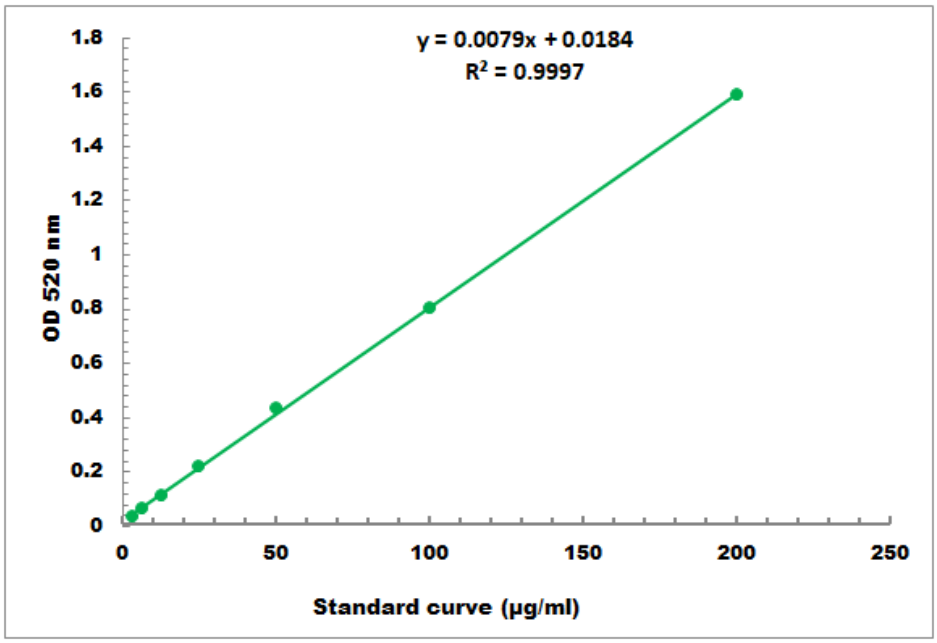
$V_{Standard}$: the volume of standard, 0.05 ml;

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

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES