Proline Microplate Assay Kit

Instructions for Use | Cat. No.: ARS6727

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

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

1. 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 Colorimetric 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.

2. Kit Components

Component	Volume	Storage
96-WellMicroplate	1 plate	-
Assay Buffer	30 mlx 4	4 °C
Reaction Buffer	5 mL x 1	4 °C
Dye Reagent	Powderx 1	4 °C
Dye Reagent Diluent	5 mL x 1	4 °C
Standard	Powderx 1	4 °C
Plate Adhesive Strips	3 strips	-
Technical Manual	1 Manual	-

3. 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. Hot air circulating oven

4. Reagent Preparation

Standard: Briefly centrifuge prior to opening. Dissolve in 1 mL distilled water to generate 2 mg/ml of standard stock solution , store at 4°C for 2 weeks or -20°C for 3-4 month safter reconstitution. Then dilute to 200 μg/ml standard top solution by adding 0.1 mL stock solution into 0.9 mL distilled water.Perform 2 -fold serial dilutions of the top standard solution using distilled water to make the standard curve.The concentration of standard curve could be 200/100/50/25/12.5/6.25/3.12 μg/ml.

Dye Reagent: Add 5 mL Dye Reagent Diluent intoDye Reagent bottle, heat to dissolve. Store at 4°C for 1 day or -20°C for 1-2 weeks after reconstitution.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

5. 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 10000 g for 1 0 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 10000 g 4°C for 1 0 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 Bufferinto the micro centrifuge tube , mix;put it into boiling water bath for 10 minutes ;centrifuged at 10000 g for 10 minutes , take the supernatant into a new centrifuge tube for detection.

6. Assay Procedure

Add following reagents in to 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 hot air circulating oven,90 °C for 20minutes, mix, record absorbance measured at 520 nm.			

Note:

*Reagents must be added sequentially and should not be premixed prior to addition.

**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.

7. Calculation

1. Calculate the sample concentration in ASSAY PROCEDURE according to the slope of

the standard curve

$$C = \frac{(OD_{Sample} - OD_{Blank}) - Intercept}{Slope} \times n \ (\mu g/ml)$$

Calculate the initial concentration according to sample preparation procedure .

2. According to one point of the standard OD and concentration

2.1 According to the protein concentration of sample

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

2.2According to the quantity of cells or bacteria

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

2.3 According to the weight of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times W \times (V_{Sample} / V_{Assay})} \ (\mu g/g)$$

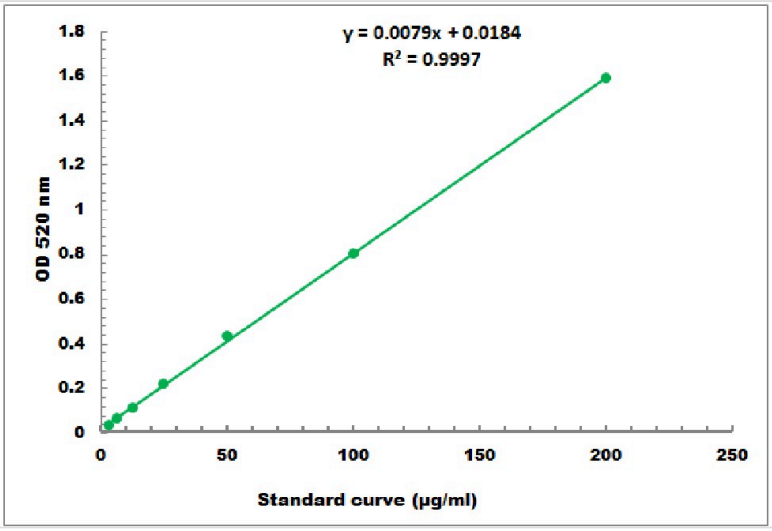
2.4According to the volume of sample

$$C = \frac{(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})}{(OD_{Standard} - OD_{Blank}) \times V_{Sample}} \ (\mu g/ml)$$

Item	Definition
Slope	the absorbance slope of standard curve
n	the dilution factor
$C_{Standard}$	the standard concentration, mg/ml
$V_{Standard}$	the volume of standard in assay procedure, μl
V_{Sample}	the volume of sample in assay procedure, μl
V_{Assay}	the volume of Assay Buffer, μl
$C_{Protein}$	the sample protein concentration, mg/ml
W	the weight of sample, g
N	the quantity of cell or bacteria, 10^4

8. 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