Proline Assay Kit

Proline Assay Kit is a detection kit for the quantification of Proline Content in urine, serum, plasma, tissue extracts, cell lysate and cell culture supernatants.

Catalog number: ARG82030

Package: 96 wells

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

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: 9F.-7, No. 12, Taiyuan 2nd St., Zhubei City,

Hsinchu County 302082, Taiwan

Phone: +886 (3) 621 8100

Fax: +886 (3) 553 0266

Email: info@arigobio.com

INTRODUCTION

Proline (symbol Pro or P) is a proteinogenic amino acid that is used in the biosynthesis of proteins. It contains an alpha-amino group (which is in the protonated NH2+ form under biological conditions), an alpha-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. It is encoded by all the codons starting with CC (CCU, CCC, CCA, and CCG).

Proline is the only proteinogenic amino acid with a secondary amine, in that the alpha-amino group is attached directly to the main chain, making the alpha carbon a direct substituent of the side chain.

L-Proline has been found to act as a weak agonist of the glycine receptor and of both NMDA and non-NMDA (AMPA/kainate) ionotropic glutamate receptors. It has been proposed to be a potential endogenous excitotoxin. In plants, proline accumulation is a common physiological response to various stresses but is also part of the developmental program in generative tissues (e.g. pollen). [Wikipedia Proline]

PRINCIPLE OF THE ASSAY

The Proline Assay Kit is used for determining Proline in various samples. Proline reacts with acidic-ninhydrin to form a stable red compound. And it can be measured at 520 nm. The Proline concentration in the sample is then determined by comparing the O.D. of samples to the standard.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Microplate	1 X 96-well plate	RT
Standard (2000 µg)	1 vial (Lyophilized)	4°C
Assay Buffer	4 X 30 ml (Ready to use)	4°C
Reaction Buffer	5 ml (Ready to use)	4°C
Reaction Dye	1 vial (Lyophilized)	4°C
Reaction Dye Diluent	5 ml (Ready to use)	4°C
Plate sealer	3 strips	RT

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 520 nm
- Pipettes and pipette tips
- Deionized or distilled water
- Convection oven (50°C, 90°C)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Reaction Dye should be store at 4°C and protect from light.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g at 2-8°C. Collect serum and assay immediately or aliquot and store samples at-20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at-20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Urine- Collect the urine by micturating directly into a sterile container. Remove impurities by centrifugation at 10,000 x g for 1 min. Collect the supernatants and assay immediately or aliquot and store samples at-20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Sample Preparation (for liquid samples):

1. Add 0.1 ml of liquid samples (serum, plasma or urine) into 0.9 ml of Assay buffer in a microcentrifuge tube, mix well.
2. Samples can be heated for 10 minutes at boiling water bath or tube heating block before centrifuge to extract the proline adequately.
3. Centrifuge the tube 10000 X g at 4 °C for 10 minutes, collect the supernatant into a new tube and keep it on ice before assay.

Cell lysate- Collect cell in a centrifuge tube, wash cells 1-2X by PBS. Discard the supernatant after centrifugation, add 1 ml of Assay buffer per 5×10^6 cell in the tube. And then sonicate samples (set with power 20%, sonicate for 3 sec. and interval for 10 sec., repeat 30 times). Samples can be heated for 10 minutes at boiling water bath or tube heating block before centrifuge to extract the proline adequately. Centrifuge samples 10000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at-20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

Tissue lysate- Weigh out 0.1 g of tissue, homogenize with 1 ml Assay buffer on ice. Samples can be heated for 10 minutes at boiling water bath or tube heating block before centrifuge to extract the proline adequately. Centrifuge samples 10000 X g at 4 °C for 10 minutes. Collect the supernatant into a new tube and keep it on ice before assay. Assay immediately or aliquot and store samples at -20°C or below for up to 1 month. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Reaction Dye:** Reconstitute the Reaction Dye with **5 ml** of **Reaction Dye Diluent**. Allow the Reaction Dye to heat for few minutes at around 50°C with gentle agitation to make sure the Reaction Dye is dissolved completely before use. The reconstituted Reaction Dye can be stored at 4°C for up to 1 week.
- **Standard:** Reconstitute the Standard with **1 ml** of **distilled water** to yield a stock concentration of 2000 µg/ml. Allow the Standard to sit for few minutes with gentle agitation to make sure the Standard is dissolved completely before use. The reconstituted standard stock can be aliquoted and stored at-20°C for up to 1 month. Dilute the reconstituted Standard stock at 1:9 with **distilled water** before use. (e.g.: add 0.1 ml of standard stock into 0.9 ml of distilled water, mix well.) The **working standard** solution will be **200 µg/ml**.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use.

Standards and samples should be assayed in at least duplicates.

1. Add **50 µl** per well of **diluted samples** and **standard** into the appropriate wells in the plate.
2. Add **50 µl** per well of **distilled water** into the Blank wells in the plate.
3. Add **50 µl** of **Reaction Buffer** into per wells.
4. Add **50 µl** of reconstituted **Reaction Dye** into per wells.
5. Gently tap the plate to ensure **thorough mixing**.
6. Cover the plate with plate sealer and incubate the plate at **90°C**, for **20 min** in dark.
7. Read the OD with a microplate reader at **520 nm** immediately.

Summary of Proline Assay Procedure

Reagent	Sample	Standard	Blank
Sample	50 µl	-	-
Standard	-	50 µl	-
Distilled water	-	-	50 µl
Reaction Buffer	50 µl	50 µl	50 µl
Reaction Dye	50 µl	50 µl	50 µl
Mix thoroughly, incubate the plate at 90°C for 20 min in dark.			
Read the OD with a microplate reader at 520 nm immediately.			

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standards and blank.

2. Calculation:

A. Definition:

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

$C_{Standard}$: the protein concentration, 200 μg/ml.

W: the weight of sample, g;

N: the quantity of cell or bacteria, $N \times 10^4$

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

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

V_{total}: the total volume of sample in Assay buffer, 1 ml;

V: the volume of sample, ml

B. Formula:

a). According to the protein concentration of sample

Proline (μg/mg) = $[(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times C_{Protein})]$

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

b). According to the weight of sample

Proline (μg/g) = $[(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Blank})] / [(OD_{Standard} - OD_{Blank}) \times (W \times V_{Sample} / V_{total})]$

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

c). According to the quantity of cells

Proline (μg/10^4 cell) = [($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - OD_{Blank})] / [($OD_{Standard}$- OD_{Blank}) X (N × V_{Sample} / V_{total})]

= 200 × (OD_{Sample}- OD_{Blank}) / [($OD_{Standard}$- OD_{Blank}) X N]

d). According to the volume of serum, plasma

Proline (μg/ml) = [($C_{Standard}$ × $V_{Standard}$) × (OD_{Sample} - OD_{Blank})] / [($OD_{Standard}$- OD_{Blank}) X V_{Sample} X (V/ V_{total})]

=200 X (OD_{Sample}- OD_{Blank}) / ($OD_{Standard}$- OD_{Blank})

3. Detection range:

The detection range is from 2 μg/ml to 200 μg/ml

EXAMPLE OF TYPICAL RESULT

The following data is for demonstration only and cannot be used in place of data generations at the time of assay. Please note this data is for demonstration only and this kit does not need serial diluted standard.