Product Manual

Proline Assay Kit

Catalog Number

MET-5196 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Proline, a non-essential amino acid which can be synthesized from glutamate, is used in protein biosynthesis. Proline lacks an -NH2 amino group but rather contains a secondary amine which is protonated (NH2+) under biological conditions, while the carboxyl group is deprotonated. Proline is a weak agonist substrate of the glycine receptor and of NMDA and non-NMDA (AMPA/kainate) ionotropic glutamate receptors. Clinically, a high proline diet was linked to greater chances of depression in humans in a study from 2022 on humans. Results were significant in the other organisms as well. Proline also plays a role in the generative tissues of plants such as pollen.

Cell Biolabs' Proline Assay Kit is a simple assay for measuring proline levels in biological samples. Proline levels may be quantified in a wide range of biological samples including serum and plasma. The kit has a detection sensitivity limit of 15.6 µM proline. Each kit provides sufficient reagents to perform up to 100 assays*, including standard curve and unknown samples.

Note: Each sample replicate requires 2 assays, one treated with Pyrroline-5-carboxylate reductase (+P5CR) and one without (-P5CR). The proline level is calculated from the difference in OD readings at a wavelength of 450 nm from the 2 wells.

Assay Principle

The Proline Assay Kit is a sensitive quantitative colorimetric assay for proline. P5CR enzyme converts proline and NAD+ to 1-pyrolline-5-carboxylate and NADH. In the presence of a Colorimetric Probe Mix containing WST-1 and an electron mediator, the WST-1 is converted to the formazan form (Figure 1) and the absorbance of the plate is read at 450 nm. The content of proline in the unknown samples is determined by comparison with a predetermined Proline standard curve.

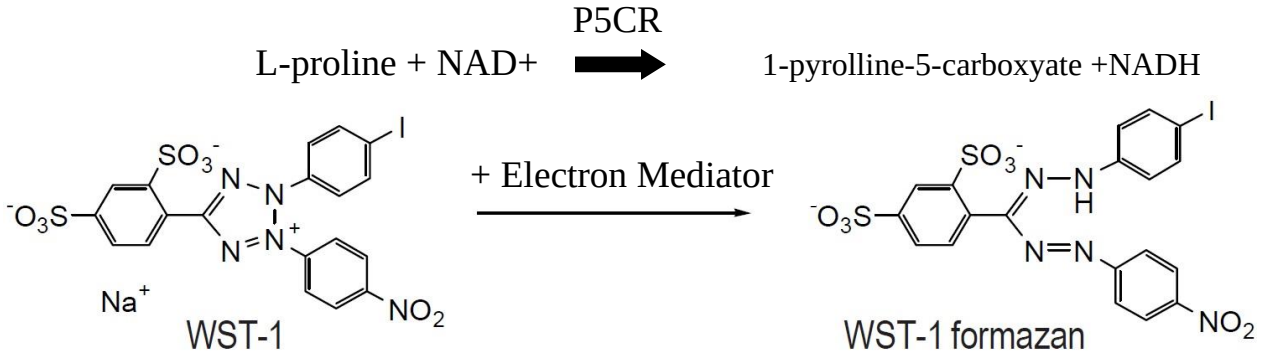

Figure 1. Assay Principle.

Related Products

1. MET-5054: L-Amino Acid Assay Kit
2. MET-5056: Branched Chain Amino Acid Assay Kit
3. MET-5071: Taurine Assay Kit
4. MET-5136: D-Amino Acid Kit (Colorimetric)
5. MET-5195: Phenylalanine Assay Kit

Kit Components (shipped on blue ice)

1. L-Proline Standard (Part No. 51961C): One 50 µL vial at 100 mM.
2. 10X Assay Buffer (Part No. 51962A): One 30 mL bottle.
3. P5CR Enzyme (Part No. 51963B): One 200 µL amber vial.
4. NAD+ (Part No. 51964D): One 400 µL vial.
5. 10X Colorimetric Probe (Part No. 51946D): Two 1 mL amber vials

Materials Not Supplied

1. Distilled or deionized water
2. Standard 96-well clear microtiter plate
3. Spectrophotometric microplate reader capable of reading absorbance at 450 nm
4. 10 kD cutoff centrifugal filter units

Storage

Upon receipt, store the P5CR enzyme at 4°C. DO NOT FREEZE THE P5CR ENZYME. Store the 10X Assay buffer at room temperature. Store all other components at -80ºC. Avoid multiple freeze/thaw cycles. The Colorimetric Probe and P5CR Enzyme are light sensitive and must be stored accordingly.

Preparation of Reagents

Note: All reagents must be brought to room temperature prior to use.

- 1X Assay Buffer: Dilute the stock 10X Assay Buffer 1:10 with deionized water for a 1X solution. Stir or vortex to homogeneity.
- Reaction Mix: Prepare a Reaction Mix by diluting the 10X Colorimetric Probe 1:10, the P5CR Enzyme 1:100, and the NAD+ 1:50 in 1X Assay Buffer. For example, for 20 assays, add 400 µL of the 10X Colorimetric Probe, 40 µL of the P5CR Enzyme, and 80 µL of NAD+ to 3480 µL of 1X Assay Buffer.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.
- Control Mix: Prepare a Control Mix by diluting the 10X Colorimetric Probe 1:10 and the NAD+ 1:50 in 1X Assay Buffer. For example, for 20 assays, add 400 µL of the 10X Colorimetric Probe and 80 µL of NAD+ to 3520 µL of 1X Assay Buffer.

  Note: Prepare only enough for immediate use by scaling the above example proportionally.

Preparation of Samples

- Cell lysates: Resuspend cells in 1X Assay Buffer. Homogenize or sonicate the cells on ice. Centrifuge 10,000 x g for 10 minutes at 4°C to remove debris. Collect the supernatant and filter the solution with a 10kD cutoff Centrifugal Filter Device to deproteinate the sample. Collect flow through. The flow through may be assayed undiluted or diluted as necessary into 1X Assay Buffer.

- Tissue lysates: Sonicate or homogenize tissue sample in 1X Assay Buffer and centrifuge at 10,000 x g for 10 minutes at 4°C. Collect the supernatant and filter the solution with a 10kD cutoff Centrifugal Filter Device to deproteinate the sample. Collect flow through. The flow through may be assayed directly or diluted as necessary into 1X Assay Buffer.

- Serum or Plasma: Deproteinate the sample by running it through a centrifugal filter unit (e.g. Amicon Ultra 0.5 mL 10K Cat. No. UFC501024) and collecting the flow through. To remove insoluble particles, centrifuge at 10,000 rpm for 5 min. Dilute the supernatant as necessary into 1X Assay Buffer just prior to performing the assay.

- Urine: To remove insoluble particles, centrifuge at 10000 x g for 10 min at 4°C. Collect the supernatant and filter the solution with a 10kD cutoff Centrifugal Filter Device to deproteinate the sample. Collect flow through. The flow through can be assayed directly or diluted as necessary into 1X Assay Buffer.

Preparation of Standard Curve

Prepare fresh Proline standards before use by diluting in distilled water according to Table 1 below.

Standard Tubes	100 mM L-Proline Solution (µL)	Distilled Water (µL)	L-Proline (µM)
1	5	495	1000
2	250 of Tube #1	250	500
3	250 of Tube #2	250	250
4	250 of Tube #3	250	125
5	250 of Tube #4	250	62.5
6	250 of Tube #5	250	31.3
7	250 of Tube #6	250	15.6
8	0	250	0

Table 1. Preparation of L-Proline Standards

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each sample, including unknowns and standards, should be assayed in duplicate or triplicate.

   Note: Each unknown sample replicate requires two paired wells, one to be treated with P5CR (+P5CR) and one without the enzyme (-P5CR) to measure endogenous background.
2. Add 50 µL of each L-proline Standard or unknown sample into wells of a 96-well microtiter plate.
3. Add 200 µL of Reaction Mix per well to standards and one half of the paired wells.
4. Add 200 µL of Control Mix per well the other half of the paired wells.
5. Incubate for 15 minutes at 37°C protected from light

   Note: This assay is continuous (not terminated) and therefore may be measured at multiple time points to follow the reaction kinetics.
6. Read absorbance of each well on a microplate reader using 450 nm as the primary wavelength.

Calculation of Results

1. Determine the average absorbance values for each sample, control, and standard.
2. Subtract the average zero standard value from itself and all standard values.
3. Graph the standard curve (see Figure 2).
4. Subtract the sample well values without P5CR (-P5CR) from the sample well values containing enzyme (+P5CR) to obtain the difference. The absorbance difference is due to the enzyme P5CR activity:

$$\Delta A = A_{(+P5CR)} - A_{(-P5CR)}$$

5. Compare the change in absorbance ΔA of each sample to the standard curve to determine and extrapolate the quantity of L-proline present in the sample. Only use values within the range of the standard curve.
6. To calculate the final L-proline concentration in the sample, take into account any prior dilutions performed.

Example of Results

The following figures demonstrate typical Proline Assay results. One should use the data below for reference only. This data should not be used to interpret actual results.

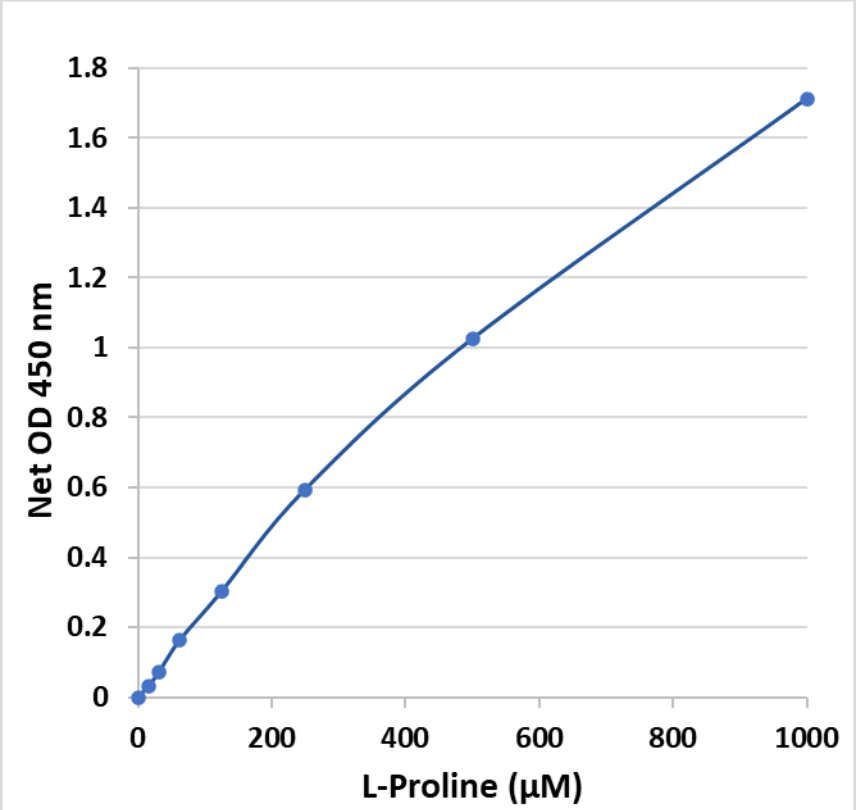

Figure 2. L-Proline Standard Curve.

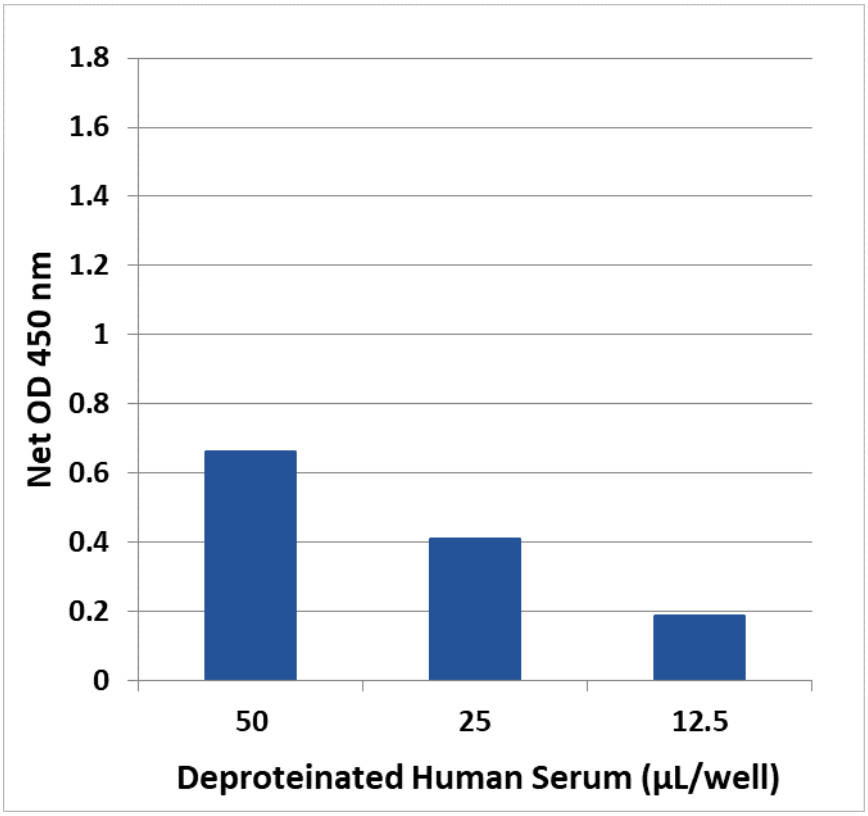

Figure 2. Detection of free proline in human serum. Human serum was deproteinated according to the preparation of samples section above.

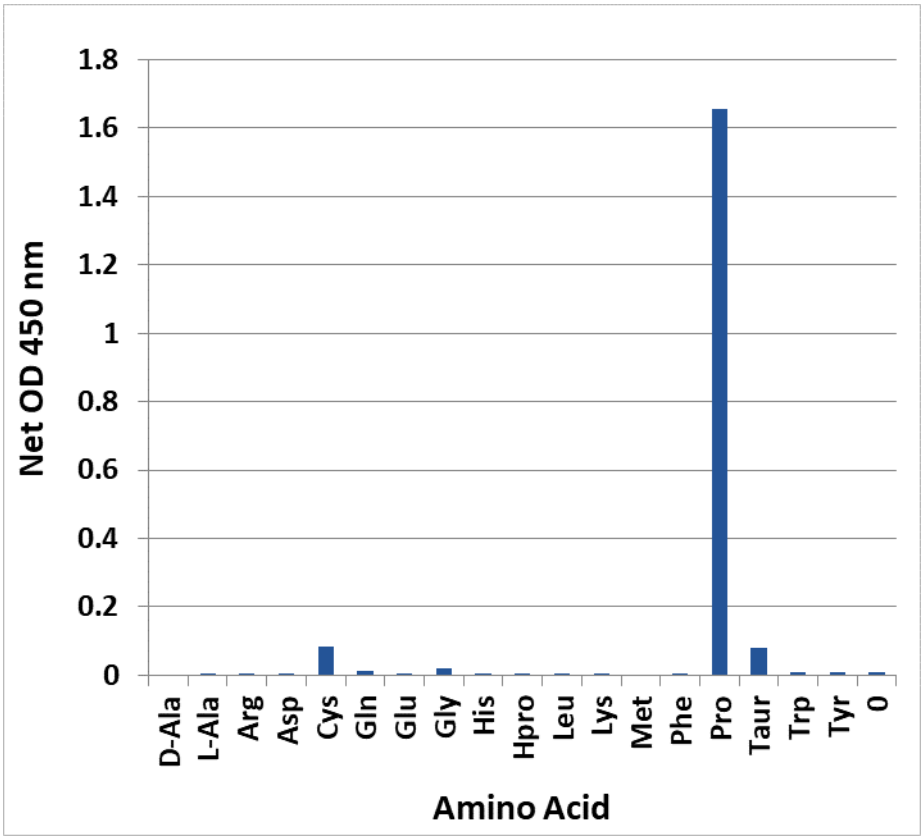

Figure 3. Specificity of L-proline Assay Kit. Reactions were performed in the presence of 500 µM D-alanine (D-Ala), L-alanine (L-Ala), L-arginine (Arg), L-asparate (Asp), L-cysteine (Cys), L-glutamine (Gln), L-glutamate (Glu), L-glycine (Gly), L-histidine (His), L-hydroxyproline (Hpro), L-leucine (Leu), L-lysine (Lys), L-methionine (Met), L-phenylalanine (Phe), L-proline (Pro) L-taurine (Taur), L-tryptophan (Trp), L-tyrosine (Tyr), or no Amino Acid (0).

References

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4. Shrestha A, Fendel A, Nguyen TH, Adebabay A, Kullik AS, Benndorf J, Leon J, and Naz AA (2022). *Plant, Cell & Environment*. **45**: 3523–3536.
5. Shrestha A, Cudjoe DK, Kamruzzaman M, Siddique S, Fiorani F, Léon J, and Naz AA (2021). *Journal of Plant Physiology*. **261**: 153414

Warranty

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