Technical Bulletin

Proline Assay Kit (Fluorometric)

Catalog Number MAK427

Product Description

Proline is a proteogenic amino acid which plays an important role in protein folding. In humans, proline can be synthesized from glutamate and arginine. Proline is one of the conditionally essential amino acids in humans. Proline is also produced by honeybees as they process nectar into honey. Thus, its content in honey is often used as an indicator of honey ripeness and sugar adulteration. For mature honey, 180 mg proline/kg of honey has been defined as an international standard. Proline also serves as a biological stress marker in plants. Unstressed plants contain approximately 0.5 µmol proline per gram of plant tissue while stressed plants can contain up to 100 times more proline than the unstressed ones.

The Proline Assay Kit provides a quick, specific and easy method for measuring proline concentration in a wide variety of samples. In this assay, proline is converted to an intermediate which subsequently reacts with a probe to produce a strong fluorometric signal (λ_{Ex} = 535 nm/λ_{Em} = 587 nm). The kit is simple, easy to use, sensitive and suitable for use in high-throughput applications. The method can detect as low as 5 pmol of proline per well.

The kit is suitable for the measurement of proline in biological samples such as serum, honey, and mammalian and plant tissue. The kit can also be used in the analysis of honey maturation and in assaying proline concentration as a stress factor in plants.

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Proline Assay Buffer 25 mL
  Catalog Number MAK427A
- Proline Enzyme Mix 1 vial
  Catalog Number MAK427B
- Proline Cofactor Mix 1 vial
  Catalog Number MAK427C
- Proline Developer Mix 1 vial
  Catalog Number MAK427D
- Probe 500 µL
  Catalog Number MAK427E
- Proline Standard 1 vial
  Catalog Number MAK427F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Refrigerated microcentrifuge capable of RCF ≥13,000 × g
- Dounce tissue grinder set (for tissue samples) (Catalog Number D9063 or equivalent)
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
- Glycerol (Catalog Number G7757 or equivalent)
- Liquid Nitrogen (for plant samples)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions.

Briefly centrifuge small vials prior to opening.

Proline Assay Buffer: Ready to use. Warm to room temperature prior to use. Store at 2-8 °C or -20 °C. Chill an appropriate amount of Proline Assay Buffer for use in Sample Preparation.

Proline Enzyme Mix: Reconstitute vial with 220 µL of 50% glycerol (prepare 50% glycerol with purified water) (not included). Allow to sit at room temperature for 30 minutes to prior to use to completely dissolve. Aliquot and store at -20 °C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months of reconstitution.

Proline Cofactor Mix: Reconstitute vial with 220 µL of purified water. Aliquot and store at -20 °C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months of reconstitution.

Proline Developer Mix: Reconstitute vial with 1 mL of Proline Assay Buffer. Aliquot and store at -20 °C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months of reconstitution.

Probe: Ready to use as supplied. Warm to room temperature prior to use. Store at -20 °C. Protect from light.

Proline Standard: Reconstitute the vial in 100 µL of purified water to make a 100 mM Proline Standard stock solution. Store the 100 mM Proline Standard stock solution at -20 °C, protected from light.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Plant Tissue Samples

Grind the plant tissue with liquid nitrogen. Rapidly homogenize tissue (~20 mg wet weight) in 100 µL of ice-cold Proline Assay Buffer with Dounce Tissue Homogenizer, and then keep on ice for 10 minutes.

Mammalian Tissue Samples

Rapidly homogenize tissue (~10 mg) in 100 µL of ice-cold Proline Assay Buffer with Dounce Tissue Homogenizer, and then keep on ice for 10 minutes.

Biological Samples (except Honey)

Centrifuge the Sample at 13,000 × g, 4 °C for 10 minutes to remove any precipitate from the liquid. Collect the supernatant and add 90 µL of the supernatant into a 10 kDa Spin Column such as Corning Spin-X UF concentrator. Centrifuge the Sample at 13,000 × g, 4 °C for 20 minutes and collect the filtrate for the assay. For normal human serum, average proline concentration ranges around 50-330 nM.

Honey

Weigh 10 mg of honey into a microcentrifuge tube. Add 100 µL of Proline Assay Buffer into the honey sample. Vortex to dissolve the honey into the buffer. Centrifuge the solution at 13,000 ×g, 4 °C for 10 minutes to remove any precipitate from the liquid. Collect the supernatant. Honey concentration is around 93 µg/µL in the Sample. For genuine honey, the proline concentration should be at least 180 mg/kg. Ten-fold or higher dilutions of the honey samples are recommended.

For **All Samples**

1. In a 96-well white plate, add 2-50 µL of the pretreated Sample(s). Proline concentration varies over a wide range for different samples. For unknown samples, perform a pilot experiment with several sample dilutions to ensure that the readings are within the Standard Curve range.
2. Adjust the total volume to 50 µL per well with Proline Assay Buffer.

Standard Curve Preparation

1. Prepare a 1 mM Proline Standard solution by diluting 10 µL of the 100 mM Proline Standard stock solution with 990 µL of purified water.
2. Further dilute the 1 mM Proline Standard solution from Step 1 to a 25 µM working Proline Standard solution by adding 25 µL of the 1 mM Proline Standard to 975 µL of purified water. Prepare Standards according to Table 1. Mix well.

Table 1.
Preparation of Proline Standards

Well	25 µM Proline Standard	Proline Assay Buffer	Proline (pmol/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	50
3	4 µL	46 µL	100
4	6 µL	44 µL	150
5	8 µL	42 µL	200
6	10 µL	40 µL	250

Reaction Mix

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Volume
Proline Assay Buffer	42 µL
Proline Enzyme Mix	2 µL
Proline Cofactor Mix	2 µL
Proline Developer Mix	2 µL
Probe	2 µL

2. Add 50 µL of the Reaction Mix into each well containing Standard and Sample(s). The volume of each well should be 100 µL/well. Mix well.

Measurement

Incubate the plate in the dark for 1 hour at 37 °C. Measure fluorescence (RFU) (λ_{Ex} = 535 nm/λ_{Em} = 587 nm) in a plate reader in endpoint mode.

Results

1. Subtract 0 Standard RFU readings from all Standard and Sample readings.
2. Plot the Proline Standard Curve (RFU vs pmol).
3. Check Sample readings against the Proline Standard Curve to obtain the amount of Proline in the wells (B).

Concentration of Proline in Fluid Samples (pmol/µL or µM) =

$$(B/V) \times D$$

Concentration of Proline in Tissue Samples (pmol/ µg or µmol/g) =

$$[B/(V \times T)] \times D$$

Concentration of Proline in Honey Samples (mg/kg) =

$$[B/(V \times H)] \times D \times MW$$

where

B = Amount of Proline calculated from the Standard Curve (in pmol)

V = Volume of Sample added to the well (in µL)

D = Sample dilution factor (if applicable; D = 1 for undiluted Samples)

T = Concentration of wet tissue/protein (in µg/µL)

H = Concentration of honey (in 93 µg/µL ≡ 9.3 × 10^{-8} kg/µL)

MW = Molecular weight of Proline (in 115.13 g/mol ≡ 1.1513 × 10^{7} mg/pmol)

Figure 1.
Typical Proline Standard Curve

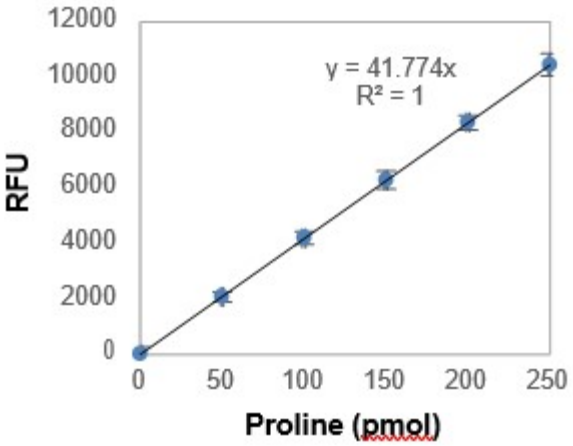


Figure 2.
Specificity of the detection of Proline over hydroxyproline and other amino acids. Other L-amino acids were tested at a 40-fold molar excess (each AA-10 nmol) vs Proline (250 pmol).

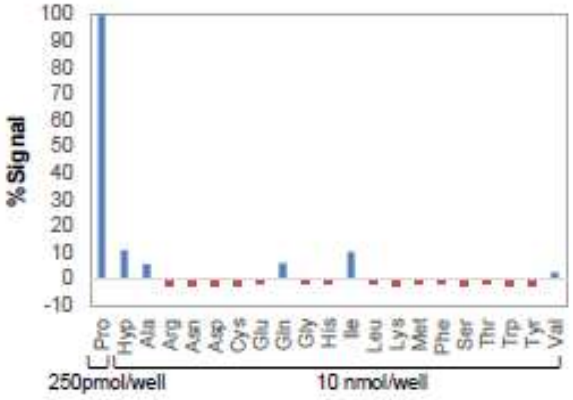
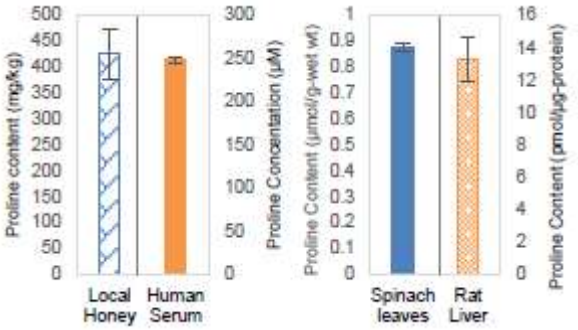


Figure 3.
Estimation of proline in local honey, human serum, spinach leaves lysate and rat liver lysate. Proline concentrations were 425.3 ± 48.9 mg/kg in local honey, 248.2 ± 3.90 µM in human serum, 0.877 ± 0.015 µmol/g-wet weight in spinach leaves, and 13.3 ± 1.4 pmol/µg-protein in rat liver lysate. Assays were performed following the kit protocol.



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