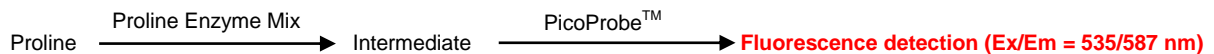# PicoProbe^{TM} Proline Assay Kit (Fluorometric)

(Catalog #K2020-100; 100 assays; Store at -20°C)

I. Introduction:

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 honey bees 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 is used as an international standard. Proline contents are measured for its biological importance in plants where it also serves as a stress marker. Unstressed plants contain approximately 0.5 µmol proline per gram of plant tissue while stressed plants can range up to 100 times more proline than the unstressed ones. BioVision's PicoProbe^{TM} Proline Assay Kit provides a quick, specific and easy method for measuring proline concentrations in a wide variety of samples. In this assay, proline is converted to an intermediate, which will further react with a probe to produce a strong fluorometric signal (Ex/Em = 535/587 nm). The kit is simple, easy to use, sensitive and high-throughput adaptable. It can detect as low as 5 pmol of proline per well.

$$\text{Proline} \xrightarrow{\text{Proline Enzyme Mix}} \text{Intermediate} \xrightarrow{\text{PicoProbe}^{TM}} \textcolor{red}{\textbf{Fluorescence detection (Ex/Em = 535/587 nm)}}$$

II. Applications:

- Measurement of proline in biological samples and honey.
- Analyzing maturation of honey.
- Assaying proline concentration as a stress factor in plants.

III. Sample Type:

- Biological fluids: serum, honey, etc.
- Mammalian and plant tissue

IV. Kit Contents:

Components	K2020-100	Cap Code	Part Number
Proline Assay Buffer	25 ml	WM	K2020-100-1
Proline Enzyme Mix	1 vial	Green	K2020-100-2
Proline Cofactor Mix	1 vial	Purple	K2020-100-3
Proline Developer Mix	1 vial	Orange	K2020-100-4
PicoProbe^{TM}	500 µl	Red	K2020-100-5
Proline Standard	1 vial	Yellow	K2020-100-6

V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (plate reader)
- 50% Glycerol
- Dounce Homogenizer (for tissue samples)
- Liquid Nitrogen (for plant samples)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay.

- **Proline Assay Buffer:** Warm to room temperature (RT) before use. Store at 4ºC or -20ºC.
- **Proline Enzyme Mix:** Reconstitute with 220 µl 50% glycerol. Incubate at RT for 30 min to dissolve the pallet. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months.
- **Proline Cofactor Mix:** Reconstitute with 220 µl dH$_2$O. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months.
- **Proline Developer Mix:** Reconstitute with 1 ml Proline Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months.
- **PicoProbe^{TM}:** Ready to use as supplied. Warm to RT before use. Store at -20°C. Keep away from light.
- **Proline Standard:** Reconstitute the vial in 100 µl of dH$_2$O to make stock 100 mM Proline Standard solution. Store the stock 100 mM Proline Standard solution at -20°C, away from light.

VII. Proline Assay Protocol:

1. Sample Preparation: For Plant Tissue Samples: Grind the plant tissue with liquid nitrogen. Rapidly homogenize tissue (~20 mg wet weight) in 100 µl ice cold Proline Assay Buffer with Dounce Tissue Homogenizer (BioVision Cat# 1998), and keep on ice for 10 min. **For Mammalian Tissue Samples:** Rapidly homogenize tissue (~10 mg) in 100 µl ice cold Proline Assay Buffer with Dounce Tissue Homogenizer (BioVision Cat. #1998), and keep on ice for 10 min. **For all Biological Samples (except Honey):** Centrifuge the Sample at 13,000 x g and 4°C for 10 min to remove the precipitate from the liquid. Collect the supernatant and add 90 µl of the supernatant into a 10 kDa Spin Column (BioVision Cat# 1997). Centrifuge the Sample at 13,000 x g and 4°C for 20 min and collect the filtrate for the assay. **For 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 x g and 4°C for 10 min to remove any precipitate from the liquid. Collect the supernatant. Honey concentration is around 93 µg/µl in the Sample.
For all Samples: In a 96-well white plate, add 2-50 µl of the pretreated Sample(s). Adjust the Sample volume to 50 µl with Proline Assay Buffer.

Notes:

a) Proline varies over a wide range for different Samples. For Unknown Samples, we recommend performing a pilot experiment with several Sample dilutions to ensure that the readings are within the Standard Curve range.

b) For normal human serum, average proline concentration ranges around 50-330 nM. For genuine honey, the proline concentration should be at least 180 mg/kg. Ten-fold or higher dilutions of the honey samples are recommended.

2. **Standard Curve Preparation:** Prepare a 1 mM Proline Standard solution by adding 10 µl of the stock 100 mM Proline Standard to 990 µl of dH$_2$O. Further dilute the 1 mM Proline Standard solution to a 25 µM working Proline Standard solution by adding 25 µl of the 1 mM Proline Standard to 975 µl of dH$_2$O. Add 0, 2, 4, 6, 8, 10 µl of the 25 µM working Proline Standard solution into a series of wells generating 0, 50, 100, 150, 200, 250 pmol of Proline/well. Adjust the volume to 50 µl/well with Proline Assay buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

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Reaction Mix

Proline Assay Buffer	42 µl
Proline Enzyme Mix	2 µl
Proline Cofactor Mix	2 µl
Proline Developer Mix	2 µl
PicoProbe™	2 µl

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Mix and 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.

4. **Measurement:** Incubate the plate in the dark for 1 hr at 37°C. Measure fluorescence (Ex/Em = 535/587 nm) in a plate reader in endpoint mode.

5. **Calculation:** Subtract 0 Standard readings from all Standard and Sample readings. Plot the Proline Standard Curve (RFU vs pmol). Check Sample readings against the Proline Standard Curve to obtain the amount of Proline in the wells (B).

Concentration of Proline in Fluid Sample = $\frac{B}{V} \times D$ = pmol/µl = µM

Concentration of Proline in Tissue Sample = $\frac{B}{V \times T} \times D$ = pmol/µg = µmol/g

Concentration of Proline in Honey Sample = $\frac{B}{V \times H} \times D \times MW$ = mg/kg

Where: B is the amount of PLP calculated from the Standard Curve (in pmol)
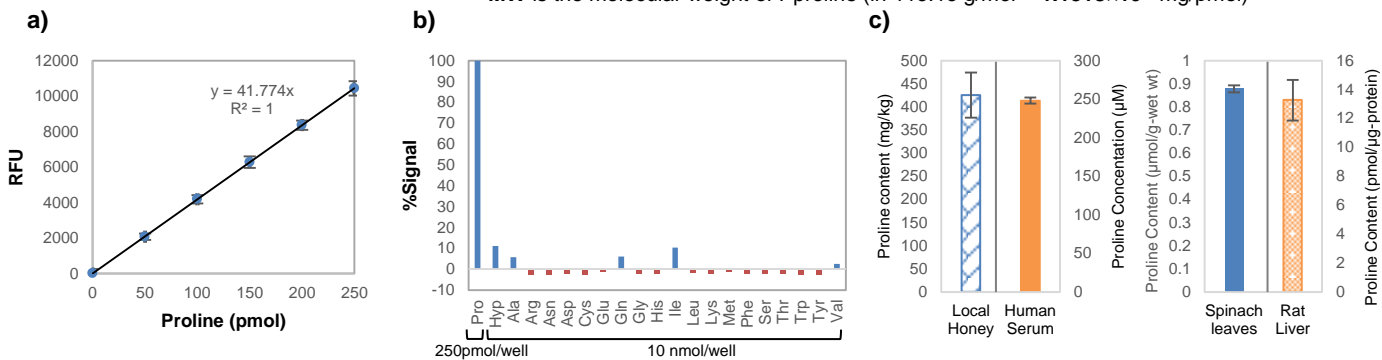**V** is the volume of Sample added to the wells (in µl)
D is the Sample dilution factor (if applicable, D= 1 for Undiluted Samples)
T is the concentration of wet tissue/protein (in µg/µl)
H is the concentration of honey (in 93 µg/µl ≡ **$9.3{\times}10^{-8}$ kg/µl**)
MW is the molecular weight of Pproline (in 115.13 g/mol ≡ **$1.1513{\times}10^{-7}$ mg/pmol**)

a)

b)

c)

Figures: (a) Proline Standard Curve. (b) 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). (c) 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 wt in spinach leaves and 13.3 ± 1.4 pmol/µg-protein in rat liver lysate. Assays were performed following the kit protocol.

VIII. **Related Products:**

DL-serine Kit (K545)	Glycine Kit (K589)	Glutamate Kit (K629)	Branch Chain Amino Acid Kit (K 564)
Alanine Kit (K652)	Phenylalanine Kit (K572)	Glutamine Assay Kit (K556)	Arginine (Colorimetric) Kit (K749)
Cysteine Kit (K558)	Tyrosine Kit (K573)	Aspartate Kit (K552)	Total Polyamine Kit (K475)
Ornithine Kit (K939)	Total D-amino acid Kit (K445)	Arginine (Fluorometric) Kit (K384)	

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FOR RESEARCH USE ONLY! Not to be used on humans.

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