

ab303731 – Proline Assay Kit (Fluorometric)

For the quantitative measurement of Proline in biological samples and honey.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab303731>

Storage and Stability

The entire ELISA kit may be stored at -20°.

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. Abcam's 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.



Materials Supplied

Item	Quantity
Proline Assay Buffer	25 ml
Proline Enzyme Mix	1 vial
Proline Cofactor Mix	1 vial
Developer Mix H	1 vial
PicoProbe I	0.4 mL
Proline Standard	1 vial

PLEASE NOTE: Developer Mix H was previously labelled as Developer Mix I and Proline Developer Mix. The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (plate reader)

- Dounce Homogenizer (for tissue samples)
- Liquid Nitrogen (for plant samples)
- 50% Glycerol

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 4°C or -20 °C.

Proline Enzyme Mix: Reconstitute with 220 µl 50% glycerol. Incubate at RT for 30 min to dissolve the pellet. 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 dH₂O. Aliquot and store at -20°C. Keep on ice while in use. Avoid freeze/thaw cycles. Use within two months.

Developer Mix H: 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 I: 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 dH₂O to make stock 100 mM Proline Standard solution. Store the stock 100 mM Proline Standard solution at -20°C, away from light.

Sample Preparation

- 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.
- 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.

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, 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, 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. 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. **▲ Note:** End user should estimate the concentration of the target protein in the test sample first and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

Assay Procedure

- Bring all reagents and samples to room temperature 30 minutes prior to the assay.
 - It is recommended that all standards and samples be run at least in duplicate.
 - A standard curve should be run for each assay.
1. 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 dH₂O. 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 dH₂O. 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.
 2. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl Reaction Mix containing:

Reaction Mix

Proline Assay Buffer	42 µl
Proline Enzyme Mix	2 µl
Proline Cofactor Mix	2 µl
Developer Mix H	2 µl
PicoProbe I	2 µl

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.

3. 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.

Calculations

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).

$$\text{Concentration of Proline in Fluid Sample} = \frac{B}{V} \times D = \text{pmol}/\mu\text{l} = \mu\text{M}$$

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

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

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 $\equiv 9.3 \times 10^{-8}$ kg/µl)

MW is the molecular weight of Proline (in 115.13 g/mol $\equiv 1.1513 \times 10^{-7}$ mg/pmol)

Technical Support

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