Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

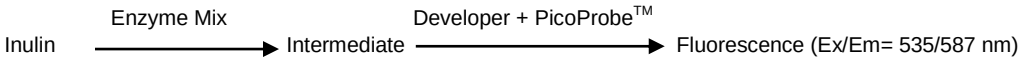
PicoProbe™ Inulin Assay Kit (Fluorometric)

11/17

(Catalog # K737-100; 100 assays; Store at -20°C)

I. Introduction:

Inulin, not to be confused with *insulin,* is an indigestible polysaccharide that is found in many plant species, where it serves as an energy storage molecule. The polymer is made up of mostly fructose units, joined primarily by β(2,1) linkages, with glucose as terminal residues. This structure renders inulin indigestible for humans, and as a result, inulin has found use as both a dietary fiber and, as it is freely filtered at the glomerulus, an indicator of renal function. Inulin is typically found in high amounts in foods such as artichoke and jicama, and is also obtained from chicory root, from which the dietary supplement is frequently isolated. As a dietary supplement, inulin is essential for maintaining intestinal health, and is often administered for this purpose. BioVision's PicoProbe™ Inulin Assay Kit allows determination of inulin content of plant material and can be assayed in biological fluids such as serum. The kit utilizes an enzymatic mechanism through which a fluorescence signal is generated proportional to the amount of inulin present in the sample. This allows the user to quantify levels of inulin down to 10 ng.



II. Applications:

- Determination of Inulin content in food and plant material
- Determination of Inulin concentration in presence of biological fluids.

III. Sample Type:

- Biological Samples: Serum, Plasma, Plant Lysates

IV. Kit Contents:

Components	K737-100	Cap Code	Part Number
Inulin Hydrolysis Buffer	25 ml	NM	K737-100-1
Inulin Reaction Buffer	25 ml	WM	K737-100-2
Inulin Enzyme Mix	1 vial	Orange	K737-100-3
Inulin Developer	1 vial	Green	K737-100-4
PicoProbe™	0.3 ml	Blue	K737-100-5
Signal Enhancer	1.5 ml	Clear	K737-100-6
Inulin Standard (1 mg/ml)	100 µl	Yellow	K737-100-7

V. User Supplied Reagents & Equipment:

- Plate reader that can be set to 37 & 45°C (Alternatively, an oven could be used for incubation purposes)
- 96-well plate (opaque black plate is recommended)
- 10 kDa spin columns for sample preparation (BV Cat. No. 1997)

VI. Storage and Reagents Preparation:

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

- **Inulin Hydrolysis Buffer and Inulin Reaction Buffer:** Store at -20°C. Warm to RT before use. Stable for two months.
- **Inulin Enzyme Mix:** Reconstitute with 1.1 ml of Inulin **Hydrolysis Buffer.** Store at -20°C. Use within two months.
- **Inulin Developer:** Reconstitute with 220 µl of **Inulin Reaction Buffer**. Mix well. Store at -20°C. Use within two months.
- **PicoProbe™ and Inulin Standard (1 mg/ml):** Ready to use. Warm to RT before use. Store at -20°C. Stable for two months.

VII. Inulin Determination Assay Protocol:

1. **Sample Preparation:** Liquid samples may be used directly. Solid samples such as food must be homogenized to a uniform consistency. Take 100 mg of a sample and homogenize in 1 ml dH_2O. Treat a 100 µl sample with Carrez Clarification reagent (K809), following the included protocol. Transfer clarified, neutralized supernatant to a separate tube. Add 2-20 µl to a well in a black 96-well plate and adjust the volume to 50 µl with Inulin Hydrolysis Buffer.

   Note:

   a. For unknown samples, we suggest doing a pilot experiment and testing several doses to ensure readings are withing the Standard Curve range. Dilute sample if necessary to obtain a result in the range of the standard curve (50-500 ng).

   b. Fructose in samples will interfere with the assay. For samples expected to have significant levels of fructose, prepare parallel sample wells(s) as background control(s). For background controls, adjust volume to **60 µl** (rather than 50 µl) with Inulin Hydrolysis Buffer.

2. **Standard Curve Preparation:** Prepare 50 µg/ml Inulin Standard as follows:

   a. To generate a 50 µg/ml Inulin Stock Solution, add 10 µl 1 mg/ml Inulin Solution to 190 µl Inulin Hydrolysis Buffer and mix well.

   b. Add 0, 2, 4, 6, 8, and 10 µl of the 50 µg/ml Inulin Stock to each well individually to generate standards of 0, 100, 200, 300, 400, and 500 ng inulin/well. Adjust the volume of each well to 50 µl with Inulin Hydrolysis Buffer.

3. **Inulin Hydrolysis Reaction:** Add 10 µl of the Inulin Enzyme Mix to the **Standard** and **Sample** wells only. *Do not add Inulin Enzyme Mix to Background Control wells*. Cover the plate, mix well, and incubate at 45°C for 30 minutes.
4. **Reaction Mix**: During incubation, mix enough reagent for the number of samples and standards to be performed: For each well (samples, background wells, and standards), prepare 50 µl Reaction Mix:

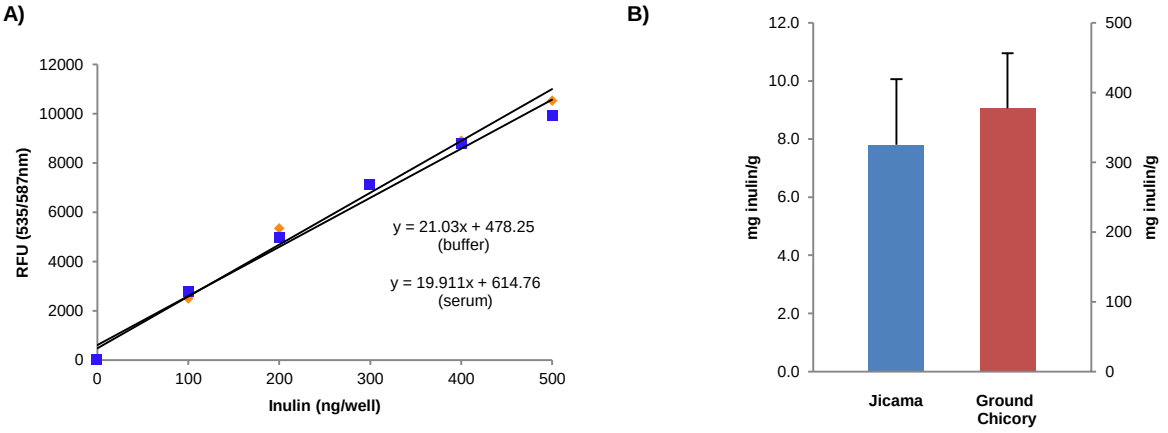
	Reaction Mix
Inulin Reaction Buffer	45 µl
Inulin Developer	2 µl
PicoProbe™	3 µl

Add 50 µl Reaction Mix to Standard, Sample, and Background Control Wells. Incubate plate at 37°C for 30 minutes.
After 30 min., add 15 µl of *Signal Enhancer and mix.

***** Enhancement linearizes the response and increases sensitivity.

5. **Measurement:** Read fluorescence in endpoint mode (Ex/Em: 535/587 nm).
6. **Calculations:** Subtract the 0 Inulin standard reading from all readings, and plot the background-subtracted Inulin standards to generate the standard curve (from 0-500 ng Inulin). For sample readings, subtract the reading obtained from the parallel reaction containing Background Control Mix. Apply the background-subtracted values to the standard curve to calculate Inulin concentration:

$$\textbf{Sample Inulin concentration } (\mathbf{C}) = \left(\frac{\text{Inulin amount from standard curve (ng)}}{\text{Vol. of sample in well(µl)}}\right) x\ Dilution\ Factor = \ (\text{ng/µl } or\ \text{µg/ml})$$



Figures. (A) No matrix effect from serum: Inulin standards were analyzed in buffer (orange diamonds) or in the presence of 5 µl undiluted serum (blue squares). Slopes were determined to be 21.0 and 19.9 RFU/ng, respectively. **(b) Inulin determination in jicama and chicory:** jicama root was processed as described. Chicory beverage powder was dissolved in water to 1 mg/ml, then treated with Carrez reagents before measurement (BV# K809). The assay was run using several (2-20 µl) concentrations of sample, and the results were plotted and slope was calculated to give the concentration of inulin present in the sample.

VIII. RELATED PRODUCTS:

Fructose Colorimetric/Fluorometric Assay Kit (K619)
Homocysteine Assay Kit (K653)
D-Fructose 1,6-bisphosphate trisodium salt (B1057)
PicoProbe Fructose-6-phosphate Fluorometric Assay Kit (K689)
PicoProbe Lactulose Fluorometric Assay Kit (K662)
PicoProbe Fructose Fluorometric Assay Kit (K611)
D-Mannitol Colorimetric Assay Kit (K644)
D-Sorbitol Colorimetric Assay Kit (K631)
Glucose Colorimetric/Fluorometric Assay Kit (K606)
β-Glucuronidase Activity Assay Kit (Fluorometric) (K514)
Starch Colorimetric/Fluorometric Assay Kit (K647)

FOR RESEARCH USE ONLY! Not to be used on humans