RayBio® PicoProbe™ Fructose Fluorometric Assay Kit

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RayBio® PicoProbe™ Fructose Fluorometric Protocol

(Cat#: 68-Fructose-S100)

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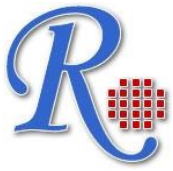

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I. INTRODUCTION

Fructose is a monosaccharide found in many foods and is one of the three most important blood sugars along with glucose and galactose. Fructose is the sweetest naturally occurring sugar, estimated to be twice as sweet as sucrose. In RayBiotech's PicoProbe™ Fructose Assay Kit, free fructose is enzymatically processed with the formation of a metabolite which reacts with the PicoProbe to generate fluorescence (Ex/Em = 535/587nm). The kit provides a simple, highly sensitive, reliable method suitable for high throughput assay of D-fructose. Glucose interference can be removed by using the Sample Cleanup Mix. The PicoProbe™ Fructose Assay Kit can detect fructose in the range of 5 to 500 picomoles/well.

II. REAGENTS

Components	XAN-S100	Cap Code	Part Number
PicoProbe Fructose Assay Buffer	25 ml	WM	Item A
PicoProbe	200 µl	Blue	Item B
Sample Cleanup Mix	Lyophilized	Orange	Item C
Conversion Enzyme	Lyophilized	Purple	Item D
Fructose Enzyme Mix	Lyophilized	Green	Item E
Fructose Substrate Mix	Lyophilized	Red	Item F
Fructose Standard (100mM)	100 µl	Yellow	Item G

III. STORAGE & HANDLING

- Store kit at –20°C and protect from light. Allow reagents to warm to room temperature and briefly centrifuge vials prior to opening.
- Keep Enzyme on ice while in use
- Read the entire protocol before the assay.

IV. REAGENT PREPARATION

- **PicoProbe:** Ready to use as supplied. Warm to > 20°C (to melt frozen DMSO) before use.
- **Sample Cleanup, Conversion Mix, Enzyme Mix:** Dissolve with 220 µl Assay Buffer. Pipette up and down to dissolve. Aliquot and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.
- **Substrate Mix:** Dissolve with 220 µl of Assay Buffer. Pipette up and down to dissolve.
    - Stable for 2 months at 4°C.
- **Fructose Standard:** Ready to use. Store at -20°C.

V. ASSAY PROCEDURE:

1. **Sample Preparation:**

   Liquid samples can be assayed directly. For tissue or cell samples: 10 - 100 mg tissue or 5 x 10^6 cells should be rapidly homogenized with 2 - 3 volumes of ice cold PBS or other buffer (pH ~8). Centrifuge at top speed for 10 min to remove insoluble materials. Add 1 – 50 µL sample into duplicate wells of a 96-well plate and bring volume to 50 µL with Assay Buffer. For unknown samples, several doses should be tested to ensure readings are within the standard curve range.

Notes:

A) Enzymes in sample may convert or consume fructose. We suggest deproteinizing samples using a perchloric acid/KOH protocol or 10 kDa molecular weight cut off spin filter to remove enzymes. Samples may be homogenized in perchloric acid, then neutralized with 10 N KOH to minimize any loss of fructose. For tissues or cells containing low levels of free fructose, minimize sample dilutions as much as possible.

B) Some biological materials in samples (NADH, NADPH, etc.) will generate background readings. You may do a sample background control (**omit Conversion Enzyme Mix** from the reaction mix) to read the background then subtract the background from sample readings.

C) Samples such as serum and urine contain high amounts of glucose which will generate high background readings. Such samples need to be pretreated with 1µL of the **Sample Cleanup Mix** for 30 min prior to analysis (dilution

effect needs to be taken into consideration later, when calculating concentrations).

D) White plates enhance the sensitivity of fluorescent assays and are recommended.

2. Standard Curve Preparations:

Dilute the Fructose Standard to 1 nmol/µL by adding 10 µL of the 100 nmol/ µL Standard to 990 µL of dH2O, mix well. Dilute further to 10 µM by adding 10µL to 990 µl of dH2O. Add 0, 2, 4, 6, 8, 10 µL into a series of standards wells on a 96 well plate. Adjust volume to 50 µL /well with Assay Buffer to generate 0, 20, 40, 60, 80, 100 pmol/well of Fructose Standard. Standard curves of more highly diluted fructose are possible if great care is taken while pipetting solutions as shown in Fig 1.

3. Develop:

Mix enough reaction mix for the number of samples and standards to be performed: For each well, prepare a total 50µL Reaction Mix containing:

	Reaction Mix	Sample Background Mix
Assay Buffer	42 µl	44 µl
Conversion Enzyme Mix	2 µl	------
Enzyme Mix	2 µl	2 µl
Substrate Mix	2 µl	2 µl
PicoProbe **	2 µl	2 µl

** To minimize baseline fluorescence and self-quenching, the PicoProbe addition should be based upon the standard curve range. For 0 - 500 pmol fructose use 2 µl/well and scale down proportionately. Add 50 µl of the Reaction Mix to each well containing the Fructose Standards and samples. Add 50 µl of the sample background mix into background control wells.

4. Incubate for 30 min at 37°C, protected from light.

5. Measure fluorescence at Ex/Em 535/587 nm.

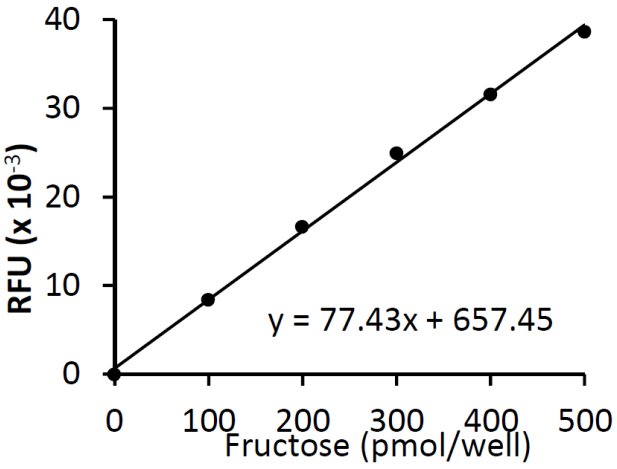
6. Calculation: Correct background by subtracting the value of the 0 Fructose Standard from all sample readings. If the sample background control reading is significant, subtract the background reading from sample readings. Plot the standard curve. Apply the corrected sample readings to the standard curve to get the amount of Fructose in the sample wells. The Fructose concentrations in the test samples is determined as follows:

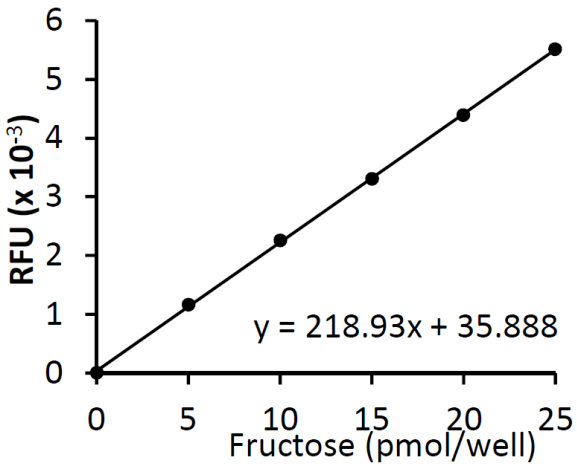
C = Ay/Sv (pmol/µl; or nmol/ml; or µM)

Where: **Ay** is the amount of fructose (pmol) in your sample from the standard curve.

Sv is the sample volume (Al) added to the sample well.

****Fructose molecular weight: 180.16.





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