

PicoProbe™ Fructose Fluorometric Assay Kit

(Catalog #K611-100; 100 assays; Store Kit at -20°C)

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 BioVision'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 = 535/587 nm). 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. Kit Contents:

Components	K611-100	Cap Code	Part Number
PicoProbe Fructose Assay Buffer	25 ml	WM	K611-100-1
PicoProbe	200 µl	Blue	K611-100-2
Sample Cleanup Mix	Lyophilized	Orange	K611-100-3
Conversion Enzyme	Lyophilized	Purple	K611-100-4
Fructose Enzyme Mix	Lyophilized	Green	K611-100-5
Fructose Substrate Mix	Lyophilized	Red	K611-100-6
Fructose Standard (100 mM)	100 µl	Yellow	K611-100-7

III. Storage and Handling:

Store kit at -20°C, protect from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep enzyme mix on ice while in use.

IV. Reagent Preparation and Storage Conditions:

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 Protocol:

1. Sample Preparation:

Liquid samples can be assayed directly. For tissue or cell samples: 10 - 100 mg tissue or 5 x 10⁶ 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 (BioVision, Cat.# K808-200) or 10 kDa molecular weight cut off spin filter (BioVision, Cat.# 1997-25) 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 1 µl 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 highly 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 10 µl 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 50 µl Reaction Mix containing:

	Reaction Mix	Sample Background
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:

$$C = Ay/Sv \text{ (pmol/µl; or nmol/ml; or µM)}$$

Where: **Ay** is the amount of fructose (pmol) in your sample from the standard curve.
Sv is the sample volume (µl) added to the sample well.
 Fructose molecular weight: 180.16.

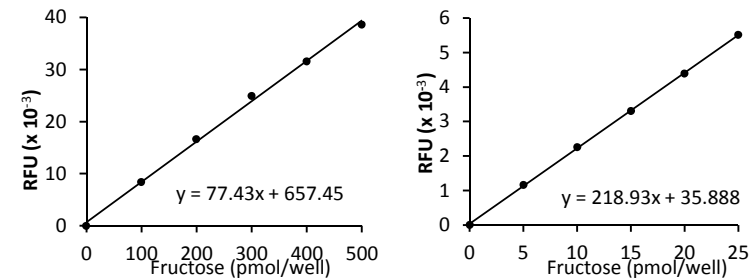


Figure 1: Fructose standard curve generated using this kit protocol

RELATED PRODUCTS:

- Fructose Assay Kit
- Glucose Assay Kits
- Glucose and Sucrose Assay Kit
- Maltose Assay Kit
- Galactose and Lactose Assay Kit
- NAD/NADH and NADP/NADPH Assay Kit
- Mono or Polysaccharide Assay Kits
- PicoProbe Fructose-6-phosphate Assay Kit
- PicoProbe Glucose-6-phosphate Assay Kit
- Glucose and Maltose Assay Kit
- Galactose Assay Kit
- Lactose Assay Kit
- Lactate Assay Kits
- Pyruvate Assay Kit

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

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	<ul style="list-style-type: none"> • Use of ice-cold assay buffer • Omission of a step in the protocol • Plate read at incorrect wavelength • Use of a different 96-well plate 	<ul style="list-style-type: none"> • Assay buffer must be at room temperature • Refer and follow the data sheet precisely • Check the wavelength in the data sheet and the filter settings of the instrument • Fluorescence: Black plates or white plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	<ul style="list-style-type: none"> • Use of an incompatible sample type • Samples prepared in a different buffer • Samples were not deproteinized (if indicated in datasheet) • Cell/ tissue samples were not completely homogenized • Samples used after multiple free-thaw cycles • Presence of interfering substance in the sample • Use of old or inappropriately stored samples 	<ul style="list-style-type: none"> • Refer data sheet for details about incompatible samples • Use the assay buffer provided in the kit or refer data sheet for instructions • Use the 10 kDa spin cut-off filter or PCA precipitation as indicated • Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope • Aliquot and freeze samples if needed to use multiple times • Troubleshoot if needed, deproteinize samples • Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> • Improperly thawed components • Use of expired kit or improperly stored reagents • Allowing the reagents to sit for extended times on ice • Incorrect incubation times or temperatures • Incorrect volumes used 	<ul style="list-style-type: none"> • Thaw all components completely and mix gently before use • Always check the expiry date and store the components appropriately • Always thaw and prepare fresh reaction mix before use • Refer datasheet & verify correct incubation times and temperatures • Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> • Use of partially thawed components • Pipetting errors in the standard • Pipetting errors in the reaction mix • Air bubbles formed in well • Standard stock is at an incorrect concentration • Calculation errors • Substituting reagents from older kits/ lots 	<ul style="list-style-type: none"> • Thaw and resuspend all components before preparing the reaction mix • Avoid pipetting small volumes • Prepare a master reaction mix whenever possible • Pipette gently against the wall of the tubes • Always refer the dilutions in the data sheet • Recheck calculations after referring the data sheet • Use fresh components from the same kit
Unanticipated results	<ul style="list-style-type: none"> • Measured at incorrect wavelength • Samples contain interfering substances • Use of incompatible sample type • Sample readings above/below the linear range 	<ul style="list-style-type: none"> • Check the equipment and the filter setting • Troubleshoot if it interferes with the kit • Refer data sheet to check if sample is compatible with the kit or optimization is needed • Concentrate/ Dilute sample so as to be in the linear range

Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.