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 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 (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 asays 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 dH_2O, mix well. Dilute further to 10 µM by adding 10 µl to 990 µl of dH_2O. 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 (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.

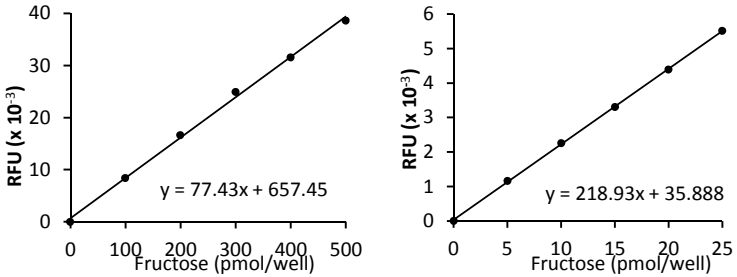

Figure 1: Fructose standard curve generated using this kit protocol

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	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates or white plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• 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.		