

PicoProbe™ Fructose-6-Phosphate Fluorometric Assay Kit

(Catalog #K689-100; 100 assays; Store Kit at -20°C)

I. Introduction:

Fructose-6-phosphate (F6P) is an important intermediate in the glycolytic pathway which leads from glucose to pyruvate. It is formed from glucose-6-phosphate and is further phosphorylated to fructose-1,6-diphosphate which is subsequently cleaved to glyceraldehyde phosphate and dihydroxyacetone phosphate. The transformation of glucose-6-phosphate is controlled by phosphoglucose isomerase, a very interesting enzyme in that it possesses multiple functions, as an isomerase, a neuroleukin, an autocrine motility factor and a differentiation and maturation mediator (1). BioVision's PicoProbe™ Fructose-6-phosphate Assay Kit is a fluorescence-based simple, highly sensitive and rapid means of quantifying F6P in a variety of samples. In the assay, F6P is converted to glucose-6-phosphate which is subsequently oxidized with the generation of a fluorescent product. The PicoProbe™ F6P Assay Kit can detect F6P in the range of 0.01 to 0.5 nmoles with detection sensitivity ~ 1 μM of F6P.

II. Kit Contents:

Components	K689-100	Cap Code	Part Number
F6P Assay Buffer	25 ml	WM	K689-100-1
F6P Probe	400 μl	Blue	K689-100-2
F6P Enzyme Mix (lyophilized)	1 vial	Purple	K689-100-3
F6P Converter (lyophilized)	1 vial	Green	K689-100-4
F6P Substrate Mix (lyophilized)	1 vial	Red	K689-100-5
F6P Standard (10 μmol, lyophilized)	1 vial	Yellow	K689-100-6

III. Storage and Handling:

Store kit at -20°C, protect from light. Warm F6P Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Keep enzyme mix on ice while in use.

VI. Reagent Preparation and Storage Conditions:

F6P Probe: Ready to use as supplied. Warm to > 20°C before use to melt frozen DMSO.

F6P Enzyme Mix, Converter, Substrate Mix: Dissolve with 220 μl F6P Assay Buffer. Pipette up and down to dissolve. Aliquot into portions and store at -20°C. Avoid repeated freeze/thaw cycles. Use within two months.

F6P Standard: Dissolve in 100 μl dH₂O to generate 100 mM (100 nmol/μl) F6P Standard solution. Keep on ice while in 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 million cells should be rapidly homogenized with 2 - 3 volumes of ice cold PBS or other buffer (pH 6.5 - 8). Centrifuge at top speed for 10 min to remove insoluble materials. Add 1 - 50 μl samples into duplicate wells of a 96-well plate and bring volume to 50 μl with Assay Buffer. For unknown samples, we suggest testing several doses of your samples to ensure readings are within the standard curve range.

Notes:

A. Enzymes in sample may convert or consume F6P. 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 F6P conversion. For tissues or cells containing low levels of free F6P (5 - 60 μM), minimize sample dilutions.

B. NADH, NADPH and glucose-6-phosphate in samples will generate background readings. If significant level of NADH, NADPH or glucose-6-phosphate is in your sample, you may do a background control (omit F6P Converter from the reaction mix) to read the background, then subtract the background from F6P readings.

C. White plates enhance the sensitivity of fluorescent assays and are highly recommended.

2. Standard Curve Preparations:

Dilute the F6P Standard to 1 nmol/μl by adding 10 μl of the 100 nmol/μl Standard to 990 μl of dH₂O, mix well. Dilute a portion further by adding 50 μl to 950 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, 0.1, 0.2, 0.3, 0.4, 0.5 nmol/well of F6P Standard.

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	Background
F6P Assay Buffer	40 μl	42 μl
F6P Enzyme Mix	2 μl	2 μl
F6P Converter	2 μl	-----
F6P Substrate	2 μl	2 μl
F6P Probe	4 μl	4 μl

Add 50 μl of the Reaction Mix to each well containing the F6P Standard and samples. Add 50 μl of the background mix into background control wells.

4. Incubate for 5 min at 37°C, protect from light.

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

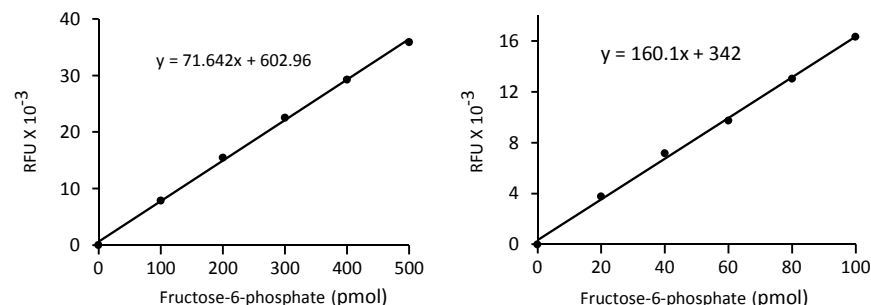
6. Calculation:

Correct background by subtracting the value of the 0 F6P blank from all readings. If background control reading is significant, subtract the background reading from sample reading. Plot the standard curve. Apply the corrected sample readings to the standard curve to get F6P amount in the sample wells. The F6P concentrations in the test samples:

$$C = Ay/Sv \text{ (nmol/}\mu\text{l; or } \mu\text{mol/ml; or mM)}$$

Where: Ay is the amount of F6P (nmol) in your sample from the standard curve.
Sv is the sample volume (μl) added to the sample well.

Fructose-6-phosphate molecular weight: 2590.81.



Fructose-6-phosphate Standard Curve Generated Using this Kit Protocol.

RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents	Cell Proliferation & Senescence Kits
Glucose and Sucrose Assay Kit	Cholesterol, LDL/HDL Assay Kits
Fructose Assay Kit	Glucose-6-Phosphate Assay Kit
Glutathione Assay Kit	Ethanol and Uric Acid Assay Kit
NAD/NADH and NADP/NADPH Assay Kit	Lactate Assay Kits
TAC Total Antioxidant Capacity	Mono or Polysaccharide Assay Kits
Malic acid Assay Kit	Pyruvate Assay Kit
Glycogen Assay Kit	Creatinine & Creatine Assay kits
Fatty Acids assay Kits	Galactose & Maltose Assay Kits
Sarcosine Assay Kit	Amino Acid Assay Kits

REFERENCES:

- 1) <http://biology.kenyon.edu/BMB/Chime2/2001/phosphoglucose%20isomerase/FRAMES/text.htm>.

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