Fructose-6-Phosphate Fluorometric Assay Kit (#BN00913)

(Catalog #BN00913; 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). Assay Genie's 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 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	BN00913	Cap Code	Part Number
F6P Assay Buffer	25 ml	WM	BN00913-1
F6P Probe	400 µl	Blue	BN00913-2
F6P Enzyme Mix (lyophilized)	1 vial	Purple	BN00913-3
F6P Converter (lyophilized)	1 vial	Green	BN00913-4
F6P Substrate Mix (lyophilized)	1 vial	Red	BN00913-5
F6P Standard (10 µmol, lyophilized)	1 vial	Yellow	BN00913-6

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 dH_2O 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 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 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 asays 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 dH_2O, mix well. Dilute a portion further by adding 50 µl to 950 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, 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.

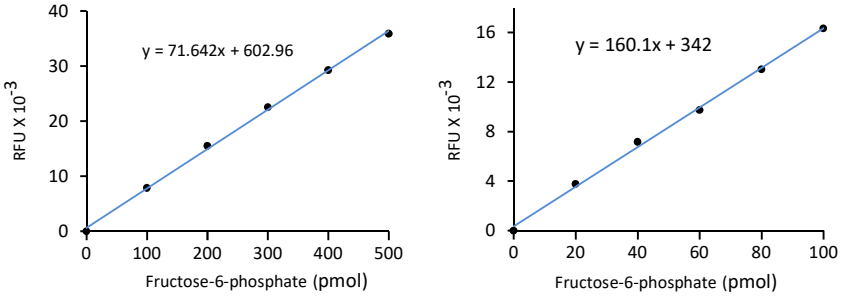
6. Calculation:

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 (nmol/µl; or µmol/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.

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.		