3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Fructose-6-Phosphate Assay Kit

Catalog Number **MAK020**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Fructose-6-phosphate (F6P) is a glycolytic pathway intermediate produced by the isomerization of glucose-6-phosphate by phosphoglucose isomerase. F6P is further phosphorylated to fructose 1,6-bisphosphate which is subsequently cleaved to glyceraldehyde phosphate and dihydroxyacetone phosphate. F6P can also be shunted from glycolysis to the non-oxidative branch of the pentose phosphate pathway as a means of generating pentose phosphates for nucleotide synthesis. F6P levels are elevated in rapidly proliferating cells such as cancer cells.

The Fructose-6-phosphate Assay Kit is a highly sensitive and simple fluorescence-based method of quantifying F6P in a variety of samples. Fructose-6-phosphate concentration is determined by a coupled enzyme reaction, which results in a fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm) product, proportional to the fructose-6-phosphate present. Typical detection range for this kit is 0.1–0.5 nmoles of F6P.

Components

The kit is sufficient for 100 assays in 96 well plates.

Fructose-6-Phosphate Assay Buffer Catalog Number MAK020A	25 mL
Fructose-6-Phosphate Probe Catalog Number MAK020B	0.4 mL
Fructose-6-Phosphate Enzyme Mix Catalog Number MAK020C	1 vl
Fructose-6-Phosphate Converter Catalog Number MAK020D	1 vl
Fructose-6-Phosphate Substrate Mix Catalog Number MAK020E	1 vl
Fructose-6-Phosphate Standard, 10 µmole Catalog Number MAK020F	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Fructose-6-Phosphate Assay Buffer – Allow buffer to come to room temperature before use.

Fructose-6-Phosphate Probe – Warm to room temperature before use to melt DMSO. Aliquot and store at –20 °C.

Fructose-6-Phosphate Enzyme Mix, Converter Mix, and Substrate Mix – Reconstitute each in 220 µL of Fructose-6-Phosphate Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Fructose-6-Phosphate Standard – Reconstitute in 100 µL of water to generate 100 mM standard solution. Mix well by pipetting, then aliquot and store at –20 °C. Keep cold while in use.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Fructose-6-Phosphate Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM Fructose-6-Phosphate standard solution with 990 µL of water to prepare a 1 mM standard solution. Take 50 µL of the 1 mM standard solution and add to 950 µL of water to make a 0.05 mM Fructose-6-Phosphate standard solution. Add 0, 2, 4, 6, 8, and 10 µl of the 0.05 mM Fructose-6-Phosphate standard solution into a 96 well plate generating 0 (blank), 0.1, 0.2, 0.3, 0.4, and 0.5 nmole/well standards. Add Fructose-6-Phosphate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid or solution samples may be assayed directly after deproteinization with a 10 kDa MWCO spin filter.

Tissue (10–100 mg) or cells (5×10^6) should be rapidly homogenized in 2–3 volumes of ice cold PBS or other buffer, pH 6.5–8. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Samples should be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction.

Bring samples to a final volume of 50 µL with Fructose-6-Phosphate Assay Buffer and transfer into duplicate wells of the 96 well plate.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Note: NADH, NADPH, and G6P in samples can generate background for the assay. To remove the effect of NADH, NADPH, or G6P background, include a blank sample for each sample by omitting the Fructose-6-Phosphate Converter in the Reaction Mix.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).
2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting. Incubate the reaction for 5 minutes at 37 °C. Protect the plate from light during the incubation.
3. Measure fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
F6P Assay Buffer	40 µL	42 µL
F6P Enzyme Mix	2 µL	2 µL
F6P Converter	2 µL	–
F6P Substrate Mix	2 µL	2 µL
F6P Probe	4 µL	4 µL

Results
Calculations
The background for the assays is the value obtained for the 0 (blank) Fructose-6-Phosphate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Fructose-6-Phosphate standards to plot a standard curve.
Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample value from the sample reading to obtain the corrected fluorescence measurement. Using the corrected fluorescence measurement, the amount of Fructose-6-Phosphate present in the sample may be determined from the standard curve.

Concentration of Fructose-6-Phosphate

$$S_a/S_v = C$$

A_y = Amount of Fructose-6-Phosphate in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added into the wells.
C = Concentration of Fructose-6-Phosphate in sample

Fructose-6-phosphate molecular weight: 260.14 g/mole

Sample Calculation
Amount of Fructose-6-Phosphate (S_a) = 0.20 nmole
Assay volume (S_v) = 50 µL

Concentration of Fructose-6-Phosphate in sample

$$0.20 \text{ nmole}/50 \text{ µL} = 0.004 \text{ nmole/µL}$$

$$0.004 \text{ nmole/µL} \times 260.14 \text{ ng/nmole} = 1.040 \text{ ng/µL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For fluorescence assays, use black plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided.
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

LS,MF,MAM 03/12-1