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Product Information

High Sensitivity Fructose Assay Kit

Catalog Number **MAK180**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Fructose is a monosaccharide naturally found in honey and fruits. Several studies have linked fructose to the development of the metabolic syndrome particularly through promoting insulin resistance in the liver. It has also been implicated in the obesity epidemic. Studies in animals have revealed high fructose intake during pregnancy and lactation may lead to metabolic dysfunctions in the mother and the newborn.[1] Fructose measurements can provide useful insights into metabolic and biochemical functions.

This kit is a highly sensitive assay for determining D-fructose levels (ranging from 20–100 pmole/well) in a variety of biological samples. Fructose concentration is determined by a coupled enzyme assay, which results in a fluorometric (λ_{ex} = 535/λ_{em} = 587 nm) product proportional to the fructose present.

Components

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

Fructose Assay Buffer Catalog Number MAK180A	25 mL
Fructose Probe Catalog Number MAK180B	0.2 mL
Sample Cleanup Mix Catalog Number MAK180C	1 vl
Conversion Enzyme Catalog Number MAK180D	1 vl
Fructose Enzyme Mix Catalog Number MAK180E	1 vl
Fructose Substrate Mix Catalog Number MAK180F	1 vl
Fructose Standard, 100 mM Catalog Number MAK180G	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate. It is recommended to use black or preferably white plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader.
- Phosphate Buffered Saline (PBS), pH ~8
- 10 kDa Molecular Weight Cut-Off (MWCO) spin filter (optional for protein-containing samples)

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the 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 Assay Buffer – Allow buffer to come to room temperature before use.

Fructose Probe – Warm to >20 °C prior to use to melt DMSO. Mix well by pipetting, then aliquot and store, protected from light and moisture, at –20 °C.

Sample Cleanup Mix, Conversion Enzyme, and Fructose Enzyme Mix – Reconstitute each in 220 µL of Fructose Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution. Keep Fructose Enzyme Mix on ice while in use.

Fructose Substrate Mix – Reconstitute with 220 µL of Fructose Assay Buffer. Mix well by pipetting, then aliquot and store at 2–8 °C. Use within 2 months of reconstitution.

Fructose Standard – Ready to use. Store at –20 °C.

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 Standards for Fluorometric Detection
Dilute 10 µL of the 100 mM (100 nmole/µL) Fructose Standard solution with 990 µL of water to prepare a 1 mM (1 nmole/µL) Standard Solution. Dilute 10 µL of the 1 mM (1 nmole/µL) Standard Solution into 990 µL of water to prepare a 10 µM (10 pmole/µL) Standard Solution. Add 0, 2, 4, 6, 8, and 10 µL of the 10 µM Standard Solution into a 96 well plate, generating 0 (blank), 20, 40, 60, 80, and 100 pmole/well standards. Add Fructose Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Liquid samples can be assayed directly.

Tissue (10–100 mg) or cells (or 5×10^6 cells) can be homogenized in 2–3 volumes of ice-cold PBS (pH ~8). Centrifuge at 13,000 × *g* for 10 minutes to remove insoluble material and collect the supernatant.

Enzymes in samples may impact fructose levels. To remove enzymes from samples, deproteinize using a 10 kDa MWCO spin filter.

To correct for background created by glucose in samples such as urine or serum, pretreat with1 µL of Sample Cleanup Mix for 30 minutes before use. The dilution effect needs to be considered for final calculations.

High levels of NADH and other biologic materials can result in a sample background. To correct for this background, include a sample blank for each sample by omitting the Conversion Enzyme. The sample blank readings can then be subtracted from the sample readings.

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

Assay Reaction
1. Add 1–50 µL of samples into duplicate wells of a 96 well plate. Bring samples to a final volume of 50 µL with Fructose Assay Buffer.

2. Set up the appropriate Reaction Mixes according to the scheme in Table 1. 50 µL of Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Sample Blank
Fructose Assay Buffer	42 µL	44 µL
Fructose Substrate Mix	2 µL	2 µL
Conversion Enzyme	2 µL	–
Fructose Enzyme Mix	2 µL	2 µL
Fructose Probe	2 µL	2 µL

3. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting and incubate the reaction for 30 minutes at 37 °C. Protect the plate from light during the incubation.

4. Measure fluorescence intensity (λ_{ex} = 535/ λ_{em} = 587 nm).

Results

Calculations

The background for the assays is the value obtained for the 0 (blank) Fructose Standard. Correct for the background by subtracting the blank standard value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate Fructose Standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the sample blank value from the sample reading to obtain the corrected fluorescence measurement. Using the corrected fluorescence measurement, determine the amount of fructose present in the sample from the standard curve.

Concentration of Fructose

$C = S_a/S_v$

where:

S_a = Amount of Fructose in unknown sample well (pmol) from standard curve

S_v = Sample volume (μL) added to well

C = Concentration of Fructose in sample (pmole/μL)

Fructose molecular weight: 180.16 g/mole

Sample Calculation

Amount of Fructose (S_a) = 25.84 pmole (from standard curve)

Sample volume (S_v) = 50 μL

Concentration of Fructose in sample:

$$25.84 \text{ pmole}/50 \ \mu\text{L} = 0.5168 \text{ pmole}/\mu\text{L}$$

$$0.5168 \text{ pmole}/\mu\text{L} \times 180.16 \text{ pg/pmole} = 93.11 \text{ pg}/\mu\text{L}$$

Reference

1. Sloboda, D.M. et al., Early life exposure to fructose and offspring phenotype: implications for long term metabolic homeostasis. J. Obes., doi: 10.1155/2014/203474 (2014).

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 or white plates with clear bottoms.
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use 10 kDa MWCO Spin Filters 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 Mixes 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 Mixes
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare Reaction Mixes 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