SIGMA-ALDRICH®

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Product Information

Fructose Assay Kit

Catalog Number **MAK265**
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.

In this assay kit, fructose is converted to β-glucose resulting in a colorimetric (570 nm)/fluorometric (λ_{ex} = 535 nm/λ_{em} = 587 nm) product proportional to fructose present. This kit has a linear range of detection between 0.2–1.0 nmole fructose for the fluorometric assay and 2–10 nmole fructose for the colorimetric assay.

This kit is suitable for use with cell and tissue culture supernatants.
Note: Not suitable for urine, plasma, nor serum

Components

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

Fructose Assay Buffer Catalog Number MAK265A	25 mL
Fructose Probe, in DMSO Catalog Number MAK265B	0.2 mL
Fructose Enzyme Mix Catalog Number MAK265C	1 vl
Fructose Converting Enzyme Catalog Number MAK265D	1 mL
Fructose Standard, (100mM) Catalog Number MAK265E	0.1 mL

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 and clear plates for colorimetric assays.
- Fluorescence or spectrophotometric multiwell plate reader

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. 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 room temperature to thaw the solution prior to use. Store protected from light at –20 °C. Use within 2 months. Upon thawing, the Fructose Probe is ready-to-use as supplied.

For the fluorescence assay, dilute an aliquot of the Fructose Probe Solution 5 to 10-fold with Assay Buffer just prior to use. This will reduce the background of the fluorescence assay.

Fructose Converting Enzyme - Remove volume needed for assay (10 µL/well). Centrifuge volume for 5 minutes at maximum speed. Remove the supernatant and reconstitute with an equal volume of Fructose Assay Buffer. Store at 2–8 °C for use within 2 months of initial thaw.
Note: Fructose Converting Enzyme is unstable when not in $(NH4)_2SO_4$ Solution.

Fructose Enzyme Mix – Reconstitute with 220 µL of Fructose Assay Buffer. Mix well by pipetting (do not vortex), then aliquot each and store, protected from light at –20 °C. Keep on ice while in use. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

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

Fructose Standards for Fluorometric Detection
Prepare a 1 nmole/µL solution as for the colorimetric assay. Dilute 10 µL of the 1 nmole/µL solution with 90 µL of the Fructose Assay Buffer to prepare a 0.1 nmole/µL solution. Add 0, 2, 4, 6, 8, and 10 µL of the 0.1 nmole/µL standard solution into a 96 well plate, generating 0 (blank), 0.2, 0.4, 0.6, 0.8, and 1.0 nmole/well standards. Add Fructose Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Both the colorimetric and fluorometric assays require 50 µL of sample for each reaction (well).

Tissue or cells (1×10^6) can be homogenized in 100 µL of the Fructose Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material. Bring samples to a final volume of 50 µL with Fructose Assay Buffer

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

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

Table 1.
Master Reaction Mix

Reagent	**Samples and Standards**
Fructose Assay Buffer	36 µL
Fructose Probe	2 µL
Fructose Enzyme Mix	2 µL
Fructose Converting Enzyme	10 µL

2. Add 50 µL of the Master Reaction Mix to each sample and standard well. Mix well using a horizontal shaker or by pipetting.

3. Incubate the plate for 2 hours at 37 °C. Protect the plate from light during the incubation.

4. For colorimetric assays, measure the absorbance at 570 nm (A_{570}). For fluorometric assays, measure fluorescence intensity (λ_{ex} = 535/λ_{em} = 587 nm).

Results

Calculations

The background for either assay is the value obtained for the 0 (assay blank) Fructose Standard. Correct for the background by subtracting the 0 (assay 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 Standards to plot a standard curve. The amount of fructose present in the samples may be determined from the standard curve.

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

The presence of glucose in a sample increases the background. If glucose is present in the sample, the glucose background can be subtracted by running a control without Fructose Converting Enzyme in the reaction. The glucose background reading can be subtracted from the sample reading that contains the Converting Enzyme to get the fructose reading.

Concentration of Fructose

$$S_a/S_v = C$$

S_a = Amount of Fructose in the unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells
C = Concentration of Fructose in sample

Fructose molecular weight: 180.16 g/mole

Sample Calculation

Amount of Fructose (S_a) = 5.84 nmole (from standard curve)
Sample volume (S_v) = 50.0 μL

Concentration of Fructose in sample

$$5.84 \text{ nmole}/50.0 \ \mu\text{L} = 0.117 \text{ nmole}/\mu\text{L}$$

$$0.117 \text{ nmole}/\mu\text{L} \times 180.16 \text{ ng/nmole} = 21.1 \text{ ng}/\mu\text{L}$$

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 plates with clear bottoms. For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	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

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