Technical Bulletin

Fructose Assay Kit

Catalogue number MAK519

Product Description

Fructose ($C_6H_{12}O_6$, also called levulose or laevulose), is a monosaccharide found in honey, tree fruits, berries, melons, and some root vegetables along with glucose and galactose. The human body can use fructose for energy, however, too much consumption may lead to high triglycerides. Simple, direct and high-throughput assays for fructose determination find wide applications. The reagent system reacts directly and specifically with fructose to form a colored product. Glucose and galactose do not interfere. The color intensity at 565 nm is directly proportional to the fructose concentration in the sample.

The linear detection range of the kit is 12 to 1000 µM fructose. The kit is suitable for determination of fructose in food, juice, beverage, other agricultural products, and biological samples such as serum, plasma, urine, saliva, milk, culture medium. It is also used to study the effects of drugs on fructose metabolism.

Components

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

- Assay Buffer 10 mL
  Catalog Number MAK519A
- Enzyme 1 vial
  Catalog Number MAK519B
- Enzyme Buffer 150 µL
  Catalog Number MAK519C
- PMS Solution 1.5 mL
  Catalog Number MAK519D
- MTT Solution 1.5 mL
  Catalog Number MAK519E
- Standard (20 mM D-Fructose) 400 µL
  Catalog Number MAK519F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (For example - multichannel pipettor)
- Spectrophotometric multi-well plate reader.
- Clear bottom 96-well plates (For example - Corning Costar). Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL centrifuge tubes
- 6 N Hydrochloric acid
- 6 N Sodium hydroxide

Precautions and Disclaimer

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.Storage/Stability

The kit is shipped on wet ice. Store all components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature.

Enzyme: Reconstitute Enzyme by adding 120 µL Enzyme Buffer to the Enzyme tube. Make sure Enzyme is fully dissolved by pipetting up and down. Store the reconstituted Enzyme at -20 °C and use within 2 months. Keep thawed tubes on ice during assay.

Note:

(1) The following substances interfere and should be avoided in sample preparation: ascorbic acid, SDS (> 0.2%), sodium azide, NP-40 (> 1%) and Polysorbate-20 (> 1%).

(2) This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to standard and samples should be quick, and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended.

Procedure

All Samples and Standards should be run in duplicates.

Sample Preparation

- Liquid Samples

  Assay can be performed directly on Samples such as serum, plasma and fruit juices can be assayed directly. Fruit juices may contain high concentrations of fructose, it is recommended to dilute juice Sample 50-fold (Dilution Factor (DF)= 50) in purified water prior to assay.

- Milk Samples

  1. Clear the milk Samples by mixing 600 µL milk with 100 µL 6 N HCl.
  2. Centrifuge at 14,000 rpm for 5 min.
  3. Transfer 300 µL supernatant into a clean tube and neutralize with 50 µL 6N NaOH. The neutralized supernatant is ready for assay (DF = 1.36).

  Transfer 20 µL of the Sample into two separate wells.

Note:

1. It is prudent to test several dilutions to determine an optimal dilution factor (DF).

Standard curve preparation:

Prepare 1000 µM Standard by mixing 12 µL 20 mM Standard and 228 µL purified water. Dilute Standards as described in the Table 1.

Table 1. Dilution of Fructose Standards

Well No.	1000 µM Fructose Standard	Purified Water (µL)	Fructose (µM)
1	100 µL	0	1000
2	60 µL	40	600
3	30 µL	70	300
4	0 µL	100	0

Transfer 20 µL of the diluted Standards into separate wells of a clear, flat-bottom 96-well plate.

Working Reagents

Keep Working Reagent protected from light. Do not expose Working Reagent to light for more than 5 minutes.

1. Mix enough reagents for the number of assays to be performed. Prepare Working Reagents according to Table 2.

Table 2. Preparation of Working Reagents for 96-well plate assay.

Reagent	Working Reagent
Assay Buffer	56 µL
Reconstituted Enzyme	1 µL
PMS Solution	14 µL
MTT Solution	14 µL

2. Add 80 µL of Working Reagent to the Standard and Sample wells.
3. Tap plate to mix briefly and thoroughly.

Measurement

1. Incubate for 60 minutes at room temperature, protected from light.
2. Read optical density at 565 nm (520-600 nm).

Results

1. Subtract the Blank value (Well #4) from the standard values.
2. Plot ΔOD of the Standard against Standard concentrations.
3. Determine slope and calculate the concentration of fructose in the Sample using the following equation:

$$(\mu M) = \frac{OD_{Sample} - OD_{Blank}}{Slope\ (\mu M^{-1})} \times DF$$

where:

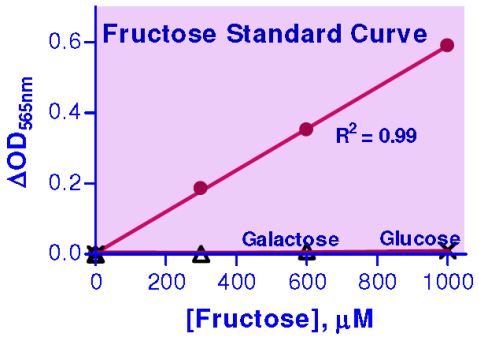
OD_{Sample} = Sample Optical Density reading

OD_{Blank} = Optical Density reading of blank (well #4)

DF = Sample Dilution Factor (DF = 1 for undiluted Samples)

Note: If the calculated fructose concentration of a sample is higher than 1000 µM, dilute sample in water and repeat the assay. Multiply result by the Dilution Factor, DF.

Figure 1.
Typical Colorimetric Fructose Standard Curve



Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

mak519pis Rev 04/23