Fructose Assay Kit

Catalog No.: abx298801

Size: 50 tests

Detection Range: 0.002 mg/ml - 2.5 mg/ml

Sensitivity: 0.002 mg/ml

Storage: Store all components at 4°C.

Application: For detection and quantification of fructose concentration in juice, honey, seminal plasma and tissue homogenates.

Introduction

Fructose is a ketonic simple sugar found commonly in plants and animals. Along with glucose and galactose, fructose is one of three major dietary monosaccharides, and is absorbed directly into the portal vein during digestion. Fructose consumption has been identified as a target for dietary research, due to its high sweetness relative to other sugars like sucrose. Fructose is a major component of semen that plays a role in the normal metabolism of spermatocytes, and thus influences fertility.

Abbexa's Fructose Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Fructose concentration. The absorbance should be measured at 285 nm. The intensity of the color is proportional to the fructose content which can then be calculated.

Kit components

1. Assay Buffer: 2 × 75 ml
2. Standard: 3 vials

Materials Required But Not Provided

1. Spectrophotometer (285 nm)
2. Double distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Cuvette (1 cm optical path)
6. Centrifuge
7. Vortex mixer
8. Water bath (100°C)
9. Ice

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Juice:** Extract fresh juice and add to a microcentrifuge tube. Centrifuge at 3500 rpm for 10 minutes. Take the supernatant, keep on ice and assay immediately.
- **Honey:** Dilute 1000-fold (for example, add 10 µl of sample to 90 µl double distilled water, then add 100 µl of the diluted sample to 900 µl of double distilled water).
- **Seminal plasma:** Collect fresh semen samples and add to a microcentrifuge tube. Place at room temperature for 1 hour, then centrifuge at 2500 rpm for 15 minutes. Take the supernatant, keep on ice and assay immediately.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 4 ml double distilled water. In an ice bath, homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Centrifuge the homogenate at 2500 rpm at 4°C for 10 min. Collect the supernatant and assay immediately. The protein concentration in the supernatant should be determined separately.

Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

We recommend carrying out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment.

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **1 mg/ml Fructose Standard Solution**: Add 10 ml of double distilled water to a vial of the Fructose Standard to prepare a 1 mg/ml Fructose Standard Solution. Prepare immediately before carrying out the assay and mix fully.

B. Assay Procedure

1. Label Blank, Standard and Sample tubes. It is recommended to measure each standard and sample in duplicate.
2. Add 0.05 ml of double distilled water to each Blank tube.

3. Add 0.05 ml of 1 mg/ml Fructose Standard Solution to each Standard tube.
4. Add 0.05 ml of sample to each Sample tube.
5. Add 3 ml of Assay Buffer to all tubes.
6. Mix fully by tapping the tube or aspiration. Incubate in a boiling water bath for 8 minutes.
7. Cool the tubes with running water. If the solution is cloudy after cooling, centrifuge at 4000 rpm for 10 minutes, and take the supernatant for detection.
8. Calibrate the spectrophotometer to zero using the blank tube. Measure the OD values of each tube (285 nm, 1cm optical path cuvette).

C. Calculation of Results

1. Juice, honey and seminal plasma samples:

To calculate the fructose content per ml of sample.

$$\text{Fructose concentration (mg/ml)} = \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{Standard}}} \times \text{C}_{\text{Standard}} \times f$$

2. Tissue homogenate samples:

To calculate the fructose content per g of protein in the sample.

$$\text{Fructose concentration (mg/g prot)} = \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{Standard}}} \times \frac{\text{C}_{\text{Standard}}}{\text{C}_{\text{Protein}}}$$

where:

$\text{OD}_{\text{Sample}}$	OD value of the sample
$\text{OD}_{\text{Standard}}$	OD value of the sample
$\text{C}_{\text{Standard}}$	The concentration of the standard (1 mg/ml)
$\text{C}_{\text{Protein}}$	The concentration of protein in the 20% tissue homogenate
f	Dilution factor of the sample before carrying out the assay