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ProductInformation

FRUCTOSE ASSAY KIT

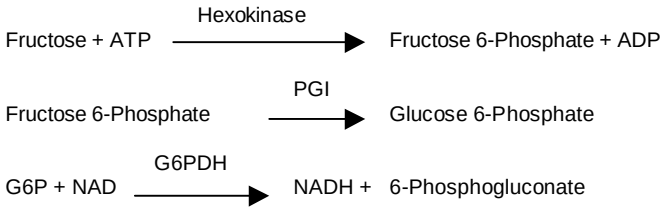
Product Code **FA-20**

TECHNICAL BULLETIN

Product Description

Enzymes, as analytical tools, have found widespread use in the food, biochemical, and pharmaceutical industry. Enzymatic methods are specific, reproducible, sensitive, rapid, and therefore ideal for analytical purposes. Due to the high specificity and sensitivity of enzymes, quantitative assays may be done on crude materials with little or no sample preparation.

This kit is for the quantitative, enzymatic determination of fructose in food and other materials.



Fructose is phosphorylated by adenosine triphosphate (ATP) in the reaction catalyzed by hexokinase. Fructose 6-phosphate is converted to glucose 6-phosphate by phosphoglucose isomerase (PGI). Glucose-6-phosphate (G6P) is then oxidized to 6-phosphogluconate in the presence of nicotinamide adenine dinucleotide (NAD) in the reaction catalyzed by glucose-6-phosphate dehydrogenase (G6PDH). During this oxidation, an equimolar amount of NAD is reduced to NADH. The consequent increase in absorbance at 340 nm is directly proportional to fructose concentration.

Reagents

1. Phosphoglucose Isomerase for Fructose Assay Kit
   (Product Code F 2668)
   Ammonium sulfate suspension containing approximately 600 U/ml of phosphoglucose isomerase (PGI) from Baker's yeast.

   The enzyme suspension is stored at 2-8 °C. DO NOT FREEZE.

2. Glucose (HK) Assay Reagent
   (Product Code G 3293)
   Reconstitute reagent vial with 50 ml of deionized water. Stopper vial and immediately mix several times by inversion. DO NOT SHAKE.

   Each vial when reconstituted with 50 ml of deionized water contains 1.5 mM NAD, 1.0 mM ATP, 1.0 U/ml hexokinase and 1.0 U/ml of glucose-6-phosphate dehydrogenase with sodium benzoate and potassium sorbate as preservatives.

   The dry reagent is stored at 2-8 °C. The reconstituted reagent is stable, in the absence of visible microbial growth, for 7 days at 18-26 °C and for 4 weeks at 2-8 °C. The reagent is not suitable for use if the absorbance of the freshly reconstituted solution measured at 340 nm vs water as reference is greater than 0.350. The reagent should be discarded if the vial exhibits caking due to possible moisture penetration, if the vial contents do not dissolve completely upon reconstitution, or if the solution appears turbid.

3. Fructose Standard
   (Product Code F 2793)
   Used as a control to ensure assay reliability. Dry reagent is stable for at least 2 years when stored desiccated at room temperature. User must determine moisture content.

Equipment Required but not Provided

1. Spectrophotometer suitable for measuring absorbance at 340 nm.
2. Cuvets
3. Pipettes capable of accurately dispensing 100 µl to 2 ml.

Precautions

Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

Sample Preparation:

Liquids: Dilute sample with deionized water to approximately 100 - 1000 µg fructose/ml.

Filter or deproteinize solution if necessary to clarify. Solutions that are strongly colored and that have a low fructose concentration should be decolorized. Carbonated or fermented products must be degassed.

Solids: Weigh out sample to nearest 0.1 mg. Extract sample with deionized water. The solution may be heated (up to about 60 °C) to aid extraction. Dilute with deionized water to approximately 100 - 1000 µg fructose/ml. Filter or deproteinize solution if necessary to clarify.

Glucose Removal:

If the sample contains a large amount of glucose (ratio of glucose to fructose is greater than 5 to 1), the glucose must be removed before assaying for fructose.[4]

In a 10 ml volumetric flask, mix:

2.0 ml 0.3 M Triethanolamine - 3 mM $MgSO_4$, pH 7.5

5.0 ml Sample (100 to 1000 µg fructose/ml)

0.1 ml Glucose Oxidase/Catalase solution (70 units of Glucose Oxidase, Product Code G 7016 and 15,000 units of Catalase, Product Code C 9631).

Bubble air through the solution for 1 hour. Check pH periodically during this time and neutralize the solution using dilute NaOH if necessary. Incubate solution in a boiling water bath for 15 minutes to inactivate enzymes. Cool solution, dilute to the 10 ml mark with deionized water and mix. Centrifuge solution to clarify, if necessary. Allow for a dilution factor of 2 in the calculations.

Fructose Determination:

Dilute sample solution to an approximate fructose concentration of 100 - 1000 µg/ml. Repeat assay and vary the sample volume if necessary to give an ΔA_{340} between 0.03 and 1.6.

1. Pipette the solutions listed in the table below into the appropriately marked test tubes.

Tube	PGI (ml)	Sample Volume (ml)	Deionized Water (ml)	Glucose Assay Reagent (ml)
PGI Blank	0.02	---	0.1	2.0
Sample Blank	---	0.1	0.02	2.0
Glucose Assay Reagent Blank	---	---	0.12	2.0
Test	0.02	0.1	---	2.0

2. Mix tubes and incubate for 15 minutes at room temperature (18-35 °C).
3. Measure the absorbance at 340 nm.

Results

Calculations:

$$A_{TOTAL\ BLANK} = (A_{SAMPLE\ BLANK} - A_{GLUCOSE\ ASSAY\ REAGENT\ BLANK}) + A_{PGI\ BLANK}$$

$$\Delta A = A_{TEST} - A_{TOTAL\ BLANK}$$

$$\text{mg fructose} = \frac{(\Delta A)(TV)(\text{Molecular Weight of Fructose})(F)}{(\varepsilon)(d)(SV)\ (\text{Conversion Factor for } \mu g \text{ to mg})}$$

$$= \frac{(\Delta A)(2.12)(180.2)(F)}{(6.22)(1)(0.1)(1000)}$$

$$= (\Delta A)(F)(0.614)$$

A = Absorbance at 340 nm d = Light path (cm)
TV = Total Assay Volume SV = Sample Volume
F = Dilution Factor from sample preparation
ε = Millimolar Extinction Coefficient for NADH at 340 nm

References

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2. Methods of Analysis of the AOAC (16th Edition) Section 28.1.17 (1995).
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4. Bergmeyer, H. U. and Bernt, E., Methods Enzym. Anal., Editor: Bergmeyer, Hans Ulrich. Publisher: Academic Press, New York. (2nd Ed.) 1177 -1178 (1974).

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