

EN

D-FRUCTOSE

REF 984302

①	3 x 16 ml Reagent 1
②	3 x 4.5 ml Reagent 2
③	3 x 4.5 ml Reagent 3

INTENDED USE

Reagent for photometric determination of D-Fructose in homogenous liquid samples using automated Thermo Scientific™ Arena™ or Gallery™ analyzer.

METHOD

Enzymatic test with hexokinase (HK), phosphoglucose isomerase (PGI) and glucose-6-phosphate dehydrogenase (G6P-DH). Method is performed at 37 °C, using 340 nm filter and 600 nm as a sidewavelength.

PRINCIPLE OF THE PROCEDURE

D-Fructose + ATP $\xrightarrow{\text{HK}}$ Fructose-6-phosphate + ADP
 D-Glucose + ATP $\xrightarrow{\text{HK}}$ Glucose-6-phosphate + ADP
 Fructose-6-phosphate $\xrightarrow{\text{PGI}}$ Glucose-6-Phosphate
 Glucose-6-phosphate + NAD⁺ $\xrightarrow{\text{G6P-DH}}$ Gluconate-6-P + NADH + H⁺

This test is not suitable for testing Glucose/Fructose ratio.

REAGENT INFORMATION

Reagent 1 (R1) 3 x 16 ml

Reagent 2 (R2) 3 x 4.5 ml

Reagent 3 (R3) 3 x 4.5 ml

Note: Labels of reagents vials have two barcodes.

For Arena analyzers, turn the short barcode on the left side to the barcode reader.

For Gallery analyzers, turn the long barcode on the right side to the reading position of the reagent rack.

Concentrations

R1	Buffer	pH 7.8
	ATP	1.7 mmol/l
	NAD	1.7 mmol/l
R2	Hexokinase (HK)	≥ 1.5 kU/l
	Glucose-6-phosphate dehydrogenase (G6P-DH). NADH to NAD converter *	≥ 1.5 kU/l
R3	Buffer	pH 7.8
	Phosphoglucose Isomerase (PGI) Stop reagent *	≥ 20 kU/l

*reagents added to stop the glucose reaction

Precautions

The reagents contain sodium azide (< 0.1 %) as preservative. Do not swallow. Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

Preparation

The reagents R1, R2 and R3 are ready-to-use.

Note: Check that there are no bubbles on the surface of the reagent when you insert vials in the analyzer.

Storage and Stability

Reagents in unopened vials are stable at 2...8 °C until the expiry date printed on the label. Do not freeze the reagents.

Reagents are stable 30 days on board.

SAMPLES

Sample Type

Food and other sample material.

Sample concentration and Arena/Gallery application

All method related details are in the separate application notes.

For the high application, the Primary dilution and the Dilution limits Low and High can be changed according to the example table below if needed.

Dilution 1+	Dilution limit (g/l)	
	Low	High
0	0.04	2.00
9	2.00	20.00
99	20.00	200.00

Sample preparation

If the sample has substances interfering the measurement, please handle it according to the following suitable preparation procedure:

- Use clear, colorless and practically neutral liquid samples directly.
- Filter or centrifuge turbid solutions.
- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples.
- Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water and filtrate, centrifuge or use Carrez clarification if necessary.
- Weigh sufficient quantity of fat containing samples into a volumetric flask (take care of the measuring range), extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter. Alternatively use Carrez clarification after extraction.
- Adjust acid samples to pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.
- Treat strongly colored samples with polyvinylpyrrolidone (PVPP e.g. 1 g/100 ml Sample).

Carrez clarification:

Weigh sufficient quantity of the sample into a 100 ml volumetric flask which contains approx. 60 ml dist. water. Subsequently carefully add 5 ml Carrez-I-solution (potassium ferrocyanide, 85 mmol = 3.60 g K₄[Fe(CN)₆] × 3 H₂O/100 ml), 5 ml Carrez-II-solution (zinc sulphate, 250 mmol = 7.20 g ZnSO₄ × 7 H₂O/100 ml) and 10 ml 0.1 M NaOH. Mix after each addition. Fill the volumetric flask with water to the mark, mix and filter.

TEST PROCEDURE

See a separate application for the Arena or Gallery analyzer.

Materials required but not provided

Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

Sugar combination standard Cat no. 984380 (one level, water based) is not included in the kit.

Calibration

Water based Sugar combination standard can be used or other. Ordering code for Sugar combination standard is 984380 (3x3 ml). The standard is ready-to-use.

Quality Control

Use quality control samples at least once a day and after each calibration and every time a new bottle of reagent is used. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

Available controls:

Sugar combination standard can be used. If Sugar combination standard is used also for calibration, an additional internal control is recommended to be used.

CALCULATION OF RESULTS

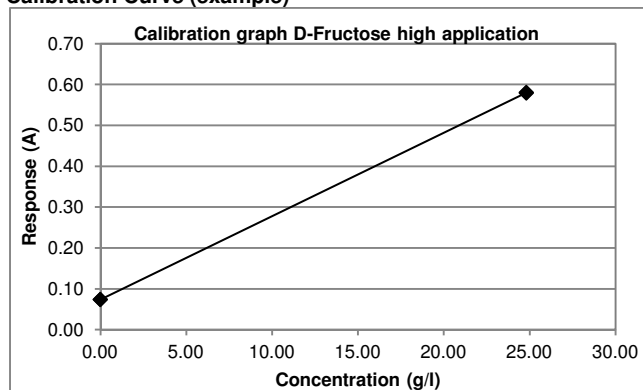
The results are calculated automatically by the analyzer using a calibration curve.

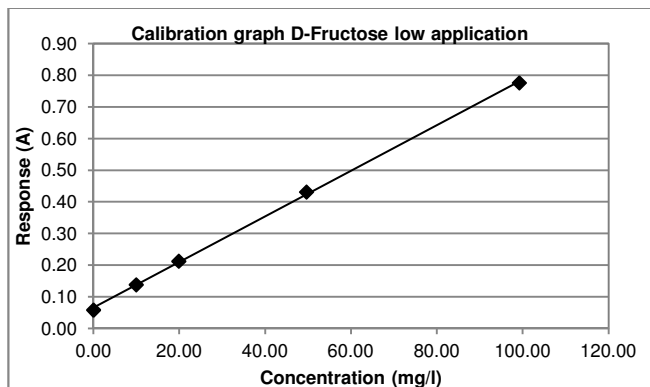
Conversion factors:

g/l × 5.5506 = mmol/l

mmol/l × 0.18016 = g/l

Calibration Curve (example)





Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

Interference

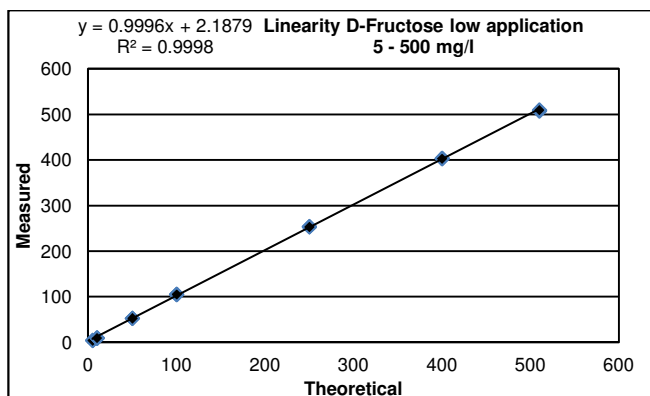
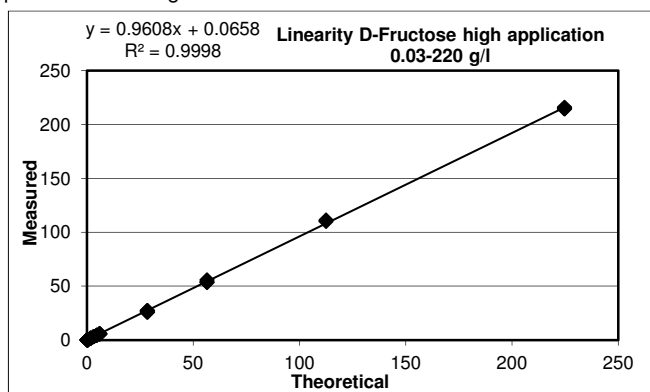
The determination is specific for D-Fructose. No interference was observed up to 100 g/l. If the D-Glucose/D-Fructose ratio is higher than 10:1, the precision of the D-Fructose determination may decrease.

MEASURING RANGE

The test has been developed to determine D-Fructose concentrations within a measuring range from 0.7 to 200 g/l (high application) and from 5 to 500 mg/l (low application).

PERFORMANCE CHARACTERISTICS

The results obtained in individual laboratories may differ from the performance data given.



Determination limit (=Test limit low)

The determination limit is the lowest concentration that can be measured quantitatively. The determination limit (measured with water based standard solution) for this method is 0.04 g/l (high application) and 5 mg/l (low application).

Precision

Gallery analyzer High application

	White wine (g/l)		Juice (g/l)		Juice (g/l)	
	N	50	N	50	N	50
	Mean	8.0	Mean	37.7	Mean	79.0
	SD	CV %	SD	CV %	SD	CV %
Within run	0.094	1.2 %	0.367	1.0 %	0.452	0.6 %
Between run	0.055	0.7 %	0.101	0.3 %	0.631	0.8 %
Total	0.109	1.4 %	0.381	1.0 %	0.776	1.0 %

Low application

	Candy (mg/l)		Candy (mg/l)		Candy (mg/l)	
	N	30	N	30	N	30
	Mean	16.10	Mean	59.02	Mean	25.00
	SD	CV %	SD	CV %	SD	CV %
Within run	0.169	1.0 %	0.133	0.2 %	0.097	0.4 %
Between run	0.071	0.4 %	0.103	0.2 %	0.103	0.4 %
Total	0.183	1.1 %	0.168	0.3 %	0.142	0.6 %

Arena shows similar performance.

OTHER REMARKS

Note that the application performance has been verified with pure chemicals dissolved in deionized water and with spiked native samples. The results obtained in individual laboratories may differ from the given performance data due to e.g. sample matrix, concentrations or analysis environment. Each laboratory is responsible to verify the method to prove the analysis performance.

WASTE MANAGEMENT

Please refer to local legal requirements. It is recommended to empty the analyzer cuvette waste bin and waste water daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions.

Note: If using reagents/solutions that react with each other, cuvette waste bin and waste water should be emptied and washed between use of these reagents.

ADDITIONAL MATERIAL

Certificate of analysis and SDS are available at www.e-labeling.eu/TSF

Applications for Gallery and Arena automated analyzers are available upon request from the local sales representative. Information in the Application note can change without prior notice.

MANUFACTURER

Thermo Fisher Scientific Oy
Ratastie 2, P.O. Box 100, FI-01621 Vantaa, Finland
Tel. +358 10 329200

CONTACT INFORMATION

www.thermoscientific.com
e-mail: system.support.fi@thermofisher.com

Date of revision

2015-07-23

CHANGES FROM PREVIOUS VERSION

Performance data added for low application.
General updates.

Template: D08896_01 © 2015 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc and its subsidiaries. This information is not intended to encourage use of these products in any manner that might infringe the intellectual property rights of others.