

EN

SUCROSE (Total Glucose)

REF 984312

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 Sucrose (Total Glucose) in homogenous liquid samples using automated Thermo Scientific™ Arena™ or Gallery™ analyzer.

METHOD

Enzymatic test with β -Fructosidase (Invertase), Hexokinase (HK) and Glucose-6-Phosphate Dehydrogenase (G6P-DH). Method is performed at 37 °C, using 340 nm filter and 600 nm as a side wavelength.

PRINCIPLE OF THE PROCEDURE

Total Glucose:

Sucrose + H₂O $\xrightarrow{\beta\text{-Fructosidase}}$ D-Glucose + D-Fructose
 D-Glucose + ATP $\xrightarrow{\text{HK}}$ Glucose-6-phosphate + ADP
 Glucose-6-phosphate + NAD⁺ $\xrightarrow{\text{G6P-DH}}$ Gluconate-6-P + NADH + H⁺

Sucrose:

The difference between D-Glucose determination with and without enzymatic conversion by β -Fructosidase indicates the value for Sucrose.

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 4.6	20 mmol/l
	β -Fructosidase		≥ 25 KU/l
R2	Buffer	pH 7.8	250 mmol/l
	ATP		10 mmol/l
	NAD		10 mmol/l
R3	Hexokinase (HK)		≥ 1.5 KU/l
	Glucose-6-phosphate dehydrogenase (G6P-DH)		≥ 1.5 KU/l

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 note.

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	0.02	1.00
9	1.00	10.00
99	10.00	100.00

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.

Thermo Scientific D-Glucose Cat no. 984304 for differentiation of Sucrose and free D-Glucose.

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.

The measured Total Glucose includes the amount of free Glucose and Glucose after splitting Sucrose by β -Fructosidase. For differentiation the free Glucose content should be measured with the Thermo Scientific D-Glucose assay in an additional run. Sucrose is calculated by subtraction of the free Glucose content from the total Glucose content. For this the ratio between the molecular weight of both sugars must be considered:
 MW (Sucrose / Glucose) = 342.30 / 180.16 = 1.90

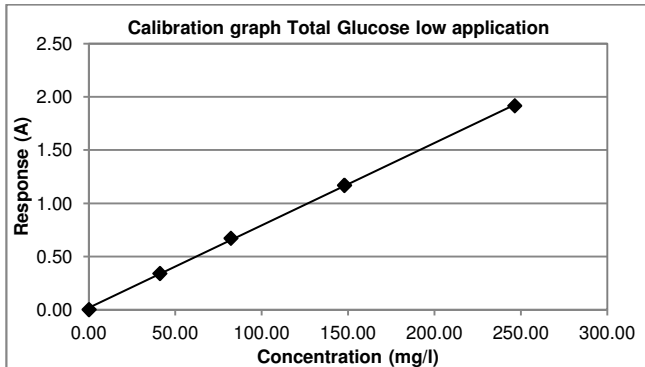
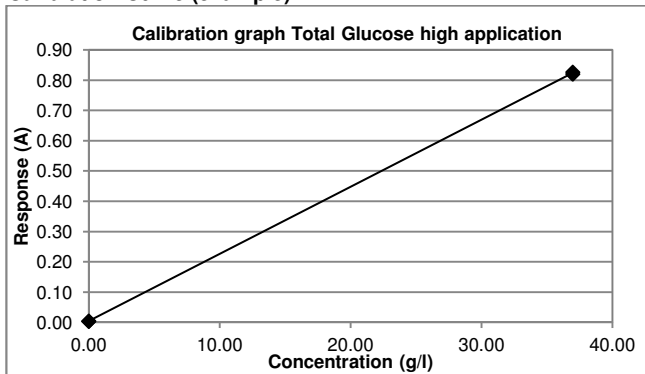
Total Sucrose (g/l) = [Total Glucose (g/l) – D-Glucose (g/l)] × 1.90

Conversion factors (sucrose):

g/l × 2.9214 = mmol/l

mmol/l × 0.3423 = g/l

Calibration Curve (example)



Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

Interference

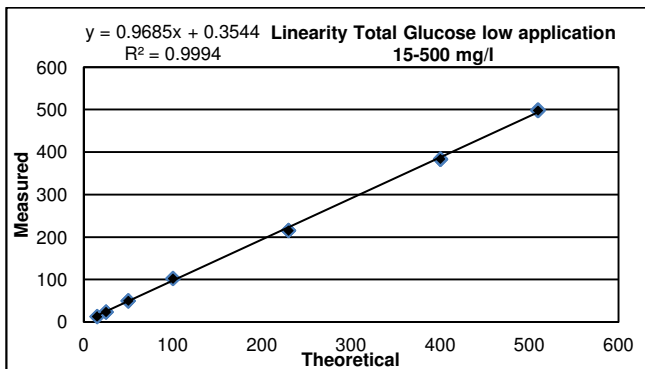
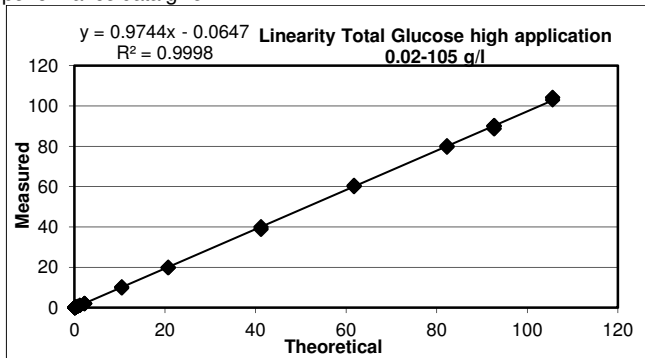
No interference has been observed.

MEASURING RANGE

The test has been developed to determine Total Glucose concentrations within a measuring range from 0.02 to 100 g/l (high application) and 15-500 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 for Total Glucose method is 0.02 g/l (high application) and 15 mg/l (low application).

Precision

Gallery analyzer

High application

	Juice (g/l)		White wine (g/l)		Juice (g/l)	
	N	50	N	50	N	50
	Mean	1.9	Mean	25.1	Mean	45.1
	SD	CV %	SD	CV %	SD	CV %
Within run	0.031	1.6 %	0.345	1.4 %	0.301	0.7 %
Between run	0.007	0.3 %	0.216	0.9 %	0.646	1.4 %
Total	0.031	1.6 %	0.406	1.6 %	0.712	1.6 %

Low application

	Candy (mg/l)		Candy (mg/l)		Candy (mg/l)	
	N	30	N	30	N	30
	Mean	304.23	Mean	298.25	Mean	219.31
	SD	CV %	SD	CV %	SD	CV %
Within run	0.936	0.3 %	0.748	0.3 %	0.492	0.2 %
Between run	1.854	0.6 %	1.394	0.5 %	0.153	0.1 %
Total	2.077	0.7 %	1.582	0.5 %	0.515	0.2 %

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.