

EN

Beta-Glucan (High MW)

REF 984305

1	3 x 19 ml Reagent R1
2	3 x 5 ml Reagent R2

INTENDED USE

Two reagent method for photometric determination of high molecular weight Barley (1,3/1,4)-Beta-D-Glucan in homogenous liquid samples using automated Thermo Scientific™ Arena™ or Gallery™ analyzer or manual spectrophotometer.

METHOD

Colorimetric method is performed at 37 °C, using 405 nm filter and 600 nm as sidewavelength.

PRINCIPLE OF THE PROCEDURE

High molecular weight Barley (1,3/1,4)-Beta-D-Glucan forms a complex with reagent R2 in buffered conditions at pH 8. Complex forming is proportional to the concentration of high molecular weight Beta-Glucan in the sample, measured at 405 nm wavelength (with sidewavelength 600 nm).

REAGENT INFORMATION

Reagent R1	3 x 19 ml	Barcode ID A15
Reagent R2	3 x 5 ml	Barcode ID A16

Note: Labels of reagent vials have two barcodes.

For Arena analyzers, turn the short barcode to the barcode reader. For Gallery analyzers, turn the long barcode to the barcode reader.

Concentrations

Dyes	
Tris Buffer	0.66 M
Detergents	0.1 %
NaOH	<0.1 %
Preservatives	<0.1 %

Precautions

R1 is hazardous.

See separate sheet inside the kit for Hazardous- and Precautions-phrases: H290, H315, H319, P280, P302 + P352, P305 + P351 + P338. EUH208: Contains Isothiazolinone compounds. May produce an allergic reaction.

R2

EUH208: Contains Isothiazolinone compounds. May produce an allergic reaction.

Exercise the normal precautions required for handling all laboratory reagents.

The product has to be disposed of as laboratory chemical in accordance with local regulations.

Preparation

Reagents are ready-to-use.

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

Storage and Stability

Reagent 2 is sensitive to light and must be stored in dark.

Reagents in unopened vials are stable at 2...8°C until the expiry date printed on the label.

Reagents in opened vials are stable at 2...8°C for one month.

Refer to the Application Notes of your analyzer for the on board stability of reagents.

SAMPLES

Sample Type

Homogenous food and beverage samples.

Sample concentration and Arena/Gallery application

All method related details are in the separate application note.

Sample preparation

Beer samples can be used directly or after degassing by shaking.

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.
- If beta-glucan has started to precipitate, heat the sample in a boiling water bath for a few minutes

TEST PROCEDURE

Automated test procedure

See a separate application for the Arena or Gallery analyzer.

Manual test procedure

Measurement is done at 405 nm with 1 cm cuvette path length. Baseline is done against air or deionized water at 37°C. Method is an end-point method, reaction time being 10 minutes. Sample/R1/R2 ratio is 1/6/0.9. Following pipetting schema is for cuvette volume 2500 µl. Manual method linearity is determined between 50-300 mg/l.

Pipette prewarmed reagents in a cuvette using the table below.

ST=Standard, S=Sample, RB=Reagent blank

	ST	S	RB
Reagent R1	1800 µl	1800 µl	1800 µl
Sample/Standard	300 µl	300 µl	----
Water	----	----	300 µl
Mix and incubate for 5 minutes at 37°C. Read the absorbance (A1) of Calibrator/Standard (AST), Sample (AS) and blank (ARB). Then add:			
Reagent R2	270 µl	270 µl	270 µl
Mix carefully. Incubate for 10 minutes at 37°C. Read the absorbance (A2) of Calibrator/Standard (AST), Sample (AS) and blank (ARB).			

Use this general formula to calculate the beta-glucan concentration mg/l:

$$\frac{(\Delta AS) \times \text{Standard value}}{(\Delta AST)}$$

$$\Delta A = (A2 - dfA1)_{\text{Sample/Standard}} - (A2 - dfA1)_{\text{Reagent blank}}$$

$$df = (\text{sample volume} + R1) / (\text{sample volume} + R1 + R2) = 0.8861$$

Note: For the samples with low beta-glucan concentration (50-100 mg/l), it is recommended to use standard concentration of 50 mg/l. For samples 100-300 mg/l, it is recommended to use standard concentration of 250 mg/l.

For concentrations higher than 300 mg/l, dilute the sample with deionized water to e.g 100 mg/l and multiply the result with the dilution factor.

For concentrations less than 50 mg/l, increased sample volumes can be tested. Note that the water volume needs to be changed accordingly. Increasing the sample volume changes the sample/reagent ratio and therefore needs to be validated by the user.

Materials required but not provided

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

Standard solutions for calibration and quality control.

Calibration

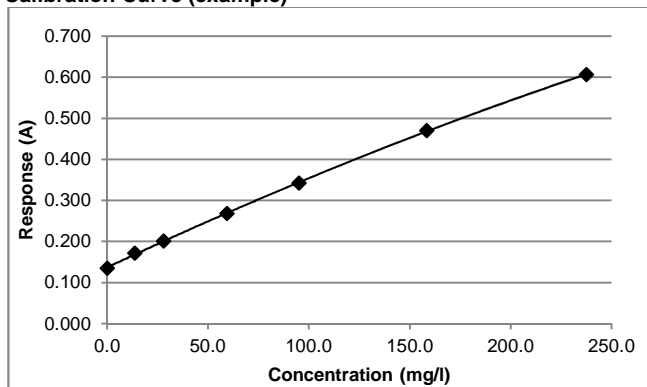
Beta-Glucan Standard Cat no 984383 can be used. Standard preparation instructions are in the insert of the Beta-Glucan Standard.

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.

CALCULATION OF RESULTS

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

Calibration Curve (example)

Calibration is measured with Gallery analyzer. Note that the calibration curve is lot and analyzer dependent.

LIMITATIONS OF THE PROCEDURE**Interference**

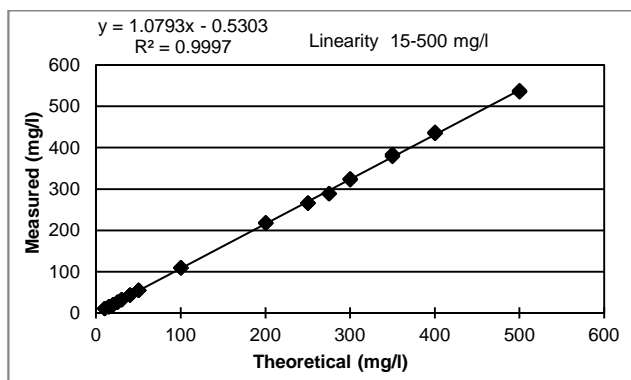
Strong sample absorbance in 405 nm (e.g. beer color) may decrease the method sensitivity or linearity.

MEASURING RANGE

The test has been developed to determine Beta-Glucan concentrations within a measuring range from 15 to 500 mg/l. Method linearity has been determined by pure chemicals dissolved in deionized water with primary dilution 1+0 from 15-300 mg/l and up to 500 mg/l with secondary dilution 1+2.

PERFORMANCE CHARACTERISTICS

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



Linearity example is measured with Gallery analyzer. Arena analyzer shows similar performance.

Precision**Gallery analyzer**

	Wort 1 (mg/l)		Wort 2 (mg/l)		Wort 3 (mg/l)	
	N	20	N	20	N	20
	Mean	195	Mean	245	Mean	91
	SD	CV %	SD	CV %	SD	CV %
Within run	0.79	0.4 %	1.34	0.5 %	0.96	1.1 %
Between run	0.17	0.1 %	0.55	0.2 %	0.13	0.1 %
Total	0.81	0.4 %	1.45	0.6 %	0.97	1.1 %

Reproducibility

Reproducibility study was performed using three Gallery analyzers for four days in different environmental conditions. Results details are seen below. Sample used was beer sample 130 mg/l.

		Gallery 1	Gallery 2	Gallery 3
30 °C / 80 % rH	n	84	81	84
	Average	126	125	124
	SD	2.42	1.86	2.20
	CV %	1.9 %	1.5 %	1.8 %
18 °C / 40 % rH	n	72	72	60
	Average	132	130	130
	SD	2.42	1.88	2.00
	CV %	1.8 %	1.4 %	1.5 %
22 °C / 40 % rH	N	66	81	69
	Average	130	131	130
	SD	3.04	1.98	2.32
	CV %	2.3 %	1.5 %	1.8 %
Total	n	84	84	84
	Average	131	128	131
	SD	2.41	2.12	1.98
	CV %	1.8 %	1.7 %	1.5 %
		n=306	n=318	n=297
		2.6 %	2.4 %	2.6 %

Similar experiment with 55 mg/l sample shows Total CV % of 3.2 (18 °C / 80 % rH), 3.2 (30 °C / 40 % rH), and 2.6 (22 °C / 40 % rH), (N=309-312).

OTHER REMARKS

Note that 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.

BIBLIOGRAPHY

1. Analytica – EBC, ISBN 978-3-418-00759-5

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
Ratatie 2, P.O. Box 100, FI-01621 Vantaa, Finland
Tel. +358 10 329200
www.thermofisher.com
www.thermofisher.com/discreteanalysis

CONTACT INFORMATION

e-mail: system.support.fi@thermofisher.com
Postal address: Thermo Fisher Scientific Oy, Ratatie 2,
P.O. Box 100, FI-01621 Vantaa, Finland

Date of revision (yyyy-mm-dd)

2017-09-29

Changes from previous version

General updates.