Megazyme

ENZYMATIC YEAST BETA-GLUCAN

ASSAY PROCEDURE

K-EBHLG 03/13

(50 Assays per Kit)

INTRODUCTION:

(1-3)-β-Glucans are widely distributed in nature, especially in algae, fungi and yeast, but also in higher plants. They serve a variety of biological functions. They form the major structural components of cell walls, they act as storage carbohydrates and they sometimes play a protective role by forming at specific sites in response to particular stimuli such as wounding.[1] Yeast β-glucan substantially enhances the function of the immune system by activating macrophages, one of the primary defences of the immune system. Literature indicates that the potent anti-tumour properties of polysaccharide fractions extracted from certain strains of mushrooms, in particular *Grifola frondosa* also known as Maitake, can be attributed to linear 6-branched (1-3)-β-glucan.[2-4] Similar properties have recently been assigned to (1-3)(1-4)-β-glucans from cereal grains (e.g. oats and barley).

The presence in wine of a particular high molecular weight (~ 800 Kd) β-glucan, produced by the fungus, *Botrytis cinerea* (grey rot) leads to clarification and filtration problems.[5] Scleroglucan (from *Sclerotium rofsii*), an industrial thickening agent with remarkable thickening properties, has a very similar structure.

Megazyme offers an acid hydrolysis/enzymic procedure (K-YBGL) for the determination of β-glucan in yeast and mushroom. That method is based on acid hydrolysis to measure total β-glucan and specific enzymic determination of α-glucan. β-Glucan is determined by difference. A totally enzymic procedure specific for β-glucan has been published by Danielson *et al.*[6] The method employs ***Lyticase*** from Sigma-Aldrich and *exo*-1,3-β-glucosidase/β-glucanase from Megazyme. This method works well, but its widespread adoption is limited by the high cost of ***Lyticase***. In this booklet we describe an alternate, simple enzymic procedure for the measurement of (1-3)(1-6)-β-glucan in yeast. The method also gives quantitation measurement of β-glucan in curdlan, laminarin and cereal β-glucan preparations. It does not give quantitative measurement of β-glucan in mushrooms, the reasons for which are currently being researched.

PRINCIPLE:

(1-3)(1-6)-β-D-Glucan, (1-3)(1-4)-β-D-Glucan and (1-3)-β-D-glucans are solubilised/hydrated in 2 N potassium hydroxide with stirring and the solution is subsequently adjusted to pH 4.0-4.5 with 1.2 M sodium acetate buffer.[7] The slurry is incubated with ***Glucazyme***™ enzyme mixture (β-glucanases, β-glucosidase and chitinase) for 16 hr at 40°C. After dilution and centrifugation, an aliquot is removed for determination of glucose with GOPOD reagent.

ACCURACY:

Standard errors of approximately < 3% are achieved routinely.

KITS:

Kits suitable for carrying out 50 assays are available from Megazyme. The kits contain the full assay method plus:

Bottle 1: ***Glucazyme***™ preparation (*exo*-1,3-β-glucanase, *endo*-1,3-β-glucanase, β-glucosidase and chitinase suspension), 2.2 mL.
Stable for > 4 years at 4°C.

Bottle 2: **GOPOD Reagent Buffer.** Buffer (48 mL, pH 7.4), *p*-hydroxybenzoic acid and sodium azide (0.4% w/v).
Stable for > 4 years at 4°C.

Bottle 3: **GOPOD Reagent Enzymes.** Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze dried powder.
Stable for > 5 years at -20°C.

Bottle 4: **D-Glucose standard solution** (5 mL, 1.5 mg/mL) in 0.2% w/v benzoic acid.
Stable for > 5 years at room temperature.

Bottle 5: **Control yeast β-glucan preparation** (~ 2 g, β-glucan content stated on the bottle label).
Stable for > 5 years at room temperature.

Bottle 6: **Control starch preparation** (~ 2 g, 96% starch dwb).
Stable for > 5 years at room temperature.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Use bottle 1 as supplied. Swirl the container contents before removing aliquots. Stand the bottle in an upright position between use. Stable for > 4 years at 4°C.

2. Dilute the contents of bottle 2 to 1.0 L with distilled or deionised water. Stable for > 2 years at 4°C.

3. Dissolve the contents of bottle 3 in the diluted contents of bottle 2 (see the preceding reagent). Divide this reagent mixture **(GOPOD reagent)** into aliquots of desired volume for storage. Stable for 2-3 months at 4°C in a dark bottle, or for > 12 months at -20°C.

4, 5 & 6. Use bottles 4, 5 & 6 as supplied. Stable for > 5 years at room temperature.

REQUIRED REAGENTS (not supplied):

1. **Sodium acetate buffer** (200 mM, pH 5.0).
   Add 11.6 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water and adjust to pH 5.0 using 4 M (16 g/100 mL) sodium hydroxide solution. Adjust the volume to 1 litre.
   Stable for ~ 1 year at 4°C.

2. **Sodium acetate buffer** (1.2 M, pH 3.8).
   Add 69.6 mL of glacial acetic acid (1.05 g/mL) to 800 mL of distilled water and adjust to pH 3.8 using 4 M sodium hydroxide. Adjust the volume to 1 litre with distilled water.
   Stable for > 2 years at room temperature.

3. **Potassium Hydroxide** (2 M).
   Add 112 g of KOH to 800 mL of distilled water and dissolve by stirring. Adjust the volume to 1 litre.
   Stable for > 2 years at room temperature.

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (round bottomed, 16 x 100 mm, 14 mL capacity).
2. Screw cap tubes, 16 x 125 mm (Fisher Scientific Cat. No. TKV-173-030B) plus caps (Cat. No. TKV-178-020V).
   Fisher Scientific, interact@fisher.co.uk.
3. Micro-pipettors, 100 µlitre (e.g. Gilson Pipetman® or Rainin EDP-2® motorised dispenser).
4. Positive displacement pipettor, e.g. Eppendorf Multipette®
   - with 5.0 mL Combitip® (to dispense 0.4 mL aliquots of 2 M KOH and 0.2 mL of ***Glucanex***™ enzyme mixture).
   - with 25 mL Combitip® [to dispense 1.5 mL aliquots of 1.2 M sodium acetate buffer (pH 3.8) and 4.0 mL of GOPOD Reagent].
5. Magnetic stirrer plus stirrer bars (5 x 15 mm).
6. Analytical balance.
7. Spectrophotometer set at 510 nm.
8. Vortex mixer.
9. Thermostated water bath set at 40°C.
10. Bench centrifuge (required speed 3,000 rpm; i.e. approx.1,500 *g*), with tube holders to accommodate 20 x 125 mm tubes.

NOTE:

With each set of determinations, include at least one control yeast preparation. Also include reagent blanks and glucose standards of 150 µg (in quadruplicate).

The **reagent blank** consists of 0.1 mL of sodium acetate buffer (200 mM, pH 5.0) + 4.0 mL glucose oxidase/peroxidase reagent.

The **D-glucose standard** consists of 0.1 mL D-glucose standard (1.5 mg/mL) + 4.0 mL glucose oxidase/peroxidase reagent.

MEASUREMENT OF 1,3:1,6-β-GLUCAN IN YEAST PREPARATIONS:

1. Mill yeast sample or other material to pass a 0.5 mm screen using a Retsch centrifugal mill, or similar.
2. Add milled sample (approximately 20 mg, weighed accurately to the nearest 0.1 mg) to a 16 x 100 mm Fisher Brand culture tube. Record the weight. Tap the tube to ensure that all of the sample falls to the bottom of the tube.
3. Add 0.4 mL of 2 M KOH and a 5 x 15 mm stirring bar. Stir the contents for 30 min in an ice water bath over a magnetic stirrer (Figure 1).
4. Add 1.6 mL of 1.2 M sodium acetate buffer (pH 3.8), mix well and then add 40 µL of ***Glucazyme***™ and cap the tubes. Continue mixing in the ice water bath for 2 min and then transfer the tubes to a water bath set at 40°C and incubate (without stirring) overnight (~ 16 h).
5. Add 10 mL of water to each tube and mix the contents thoroughly. Centrifuge the tubes at 3000 rpm in a bench centrifuge for 10 min.
6. Carefully transfer 0.1 mL aliquots of the sample in duplicate to the bottom of 16 x 120 mm tubes.
7. Add 4 mL of GOPOD reagent to each of the reaction tubes, the controls, the standards and reagent blanks, and incubate the tubes for 20 min at 40°C.
8. Read the absorbance at 510 nm of each solution against a reagent blank.

CALCULATIONS:

β-Glucan (% w/w)

$$= \Delta E \times F \times \frac{12.04}{0.1} \times \frac{100}{W} \times \frac{1}{1000} \times \frac{162}{180}$$

$$= \Delta E \times F/W \times 10.836.$$

where:

ΔE	=	Absorbance read against reagent blank.
F	=	Conversion from absorbance to µg (150 µg of D-glucose) standard divided by GOPOD absorbance of this 150 µg).
12.04/0.1	=	Volume correction (0.1 mL taken from 12.04 mL).
1/1000	=	Conversion from µg to mg.
W	=	Weight of sample analysed in mg.
100/W	=	Factor to present β-glucan as a percentage of sample weight.
162/180	=	Factor to convert from free D-glucose to anhydro-D-glucose as occurs in β-glucan.

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

REFERENCES:

1. Stone, B. A. and Clarke, A. E. (1992). In: **Chemistry and Biology of (1-3)-β-Glucans.** La Trobe University Press, Victoria, Australia.
2. Ohno, N., Suzuki, I., Oikawa, S. *et al.* (1984). Antitumour Activity and Structural Characterisation of Glucans Extracted from Cultured Fruit Bodies of *Grifola frondosa*. *Chem. Pharm. Bull.,* **32**, 1142-1151.
3. Ohno, N., Lino, K., Takahiro, T. *et al.* (1985). Structural Characterisation and Antitumour Activity of the Extracts from Matted Mycelium of Cultured *Grifola frondosa*. *Chem. Pharm. Bull.,* **33,** 3395-3401.

4. Lino, K., Ohno, N., Suzuki, I., Miyazaki, T. and Yadomae, T. (1985). Structural Characterisation of a Neutral Antitumour β-D-Glucan Extracted with Hot Sodium Hydroxide from Cultured Fruit Bodies of *Grifola frondosa*. *Carbohydrate Research*, **141**, 111-119.

5. Villettaz, J. C., Steiner, D. and Trogus, H. (1984). The use of a Beta Glucanase as an Enzyme in Wine Clarification and Filtration. *Am. J. Enol. Vitic.,* **35**, 253-256.

6. Danielson, M. E., Dauth, R., Elmasry, N. A., Langeslay, R. R., Magee, A. S. and Will, P. M. (2010). Enzymatic method to measure β-1,3-β-1,6-glucan content in extracts and formulated products (GEM assay). *J. Agric. Food Chem.,* **58,** 10305-10308.

7. McCleary, B. V. & Monaghan, D. A. (2002). Measurement of resistant starch. *J. AOAC International,* **85**, 665-675.

Figure 1. Arrangement of ice-water bath over a magnetic stirrer for dissolution / hydration of β-glucan in KOH.

Table 1. Determined β-glucan content of a number of samples using the enzymic procedure.

Sample Description	β-Glucan content (as is)	Moisture content (%)	β-Glucan content (dry weight basis)
Yeast β-glucan control (lot 90201a)	63.3	5.3	66.8
Barley β-Glucan (Lot 90801a)	90.0	7.8	97.6
Scleroglucan (Actigum CS11)	86.1	3.4	89.1
Curdlan (Lot 60201a)	87.7	10.0	97.4
Pachyman (lot 10301a)	84.0	3.0	86.6
Laminarin (*Eisenia arborea;* Tokyo Kasei)	79.4	11.5	89.7
Alpha-Cellulose	9.5	1.5	9.6
Avicel	13.9	4.3	14.5
Soluble starch (Sigma Chemical Co.)	0.32	5.5	0.34
Glycogen Type II (Sigma G-8751)	0.25	8.0	0.27

NOTE: Alpha-Cellulose and Avicel (cellulose) are only partially hydrolysed. Yeast β-glucan, barley β-glucan, scleroglucan, curdlan, pachyman and laminarin are completely hydrolysed. Hydrolysis of α-glucans (starch and glycogen) by the enzyme mixture used is minor (approx 0.3%).

Megazyme International Ireland,
Bray Business Park, Bray,
Co. Wicklow,
IRELAND.

Telephone: (353.1) 286 1220
Facsimile: (353.1) 286 1264
Internet: www.megazyme.com
E-Mail: info@megazyme.com