# β-GLUCAN
# (Mixed Linkage)

PRODUCT INSTRUCTIONS

## SKU: 700004269
## K-BGLU

05/25

(100 Manual Assays per Kit)

**AACC Method 32-23.01**
**AOAC Method 995.16**
**ICC Standard Method No. 166**
**Codex Type II Method**

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**INTRODUCTION:**

The Neogen® β-glucan assay kit (**K-BGLU**, **700004269**) is an accurate, convenient, and reliable method for the measurement of mixed-linkage β-glucan in barley, malt, wort and beer. The method is straightforward allowing ~ 50-100 samples to be assayed in a day. An assay protocol for the measurement of β-glucan in oats and oat fiber products is also provided (Streamlined method, page 4).

**PRINCIPLE:**

Samples are suspended and hydrated in a buffer solution of pH 6.5 and then incubated with purified lichenase enzyme and filtered. An aliquot of the filtrate is then hydrolysed to completion with purified β-glucosidase. The D-glucose produced is assayed using a glucose oxidase/peroxidase reagent (Scheme 1, page 15).

**ACCURACY:**

Standard errors of ± 3% are achieved routinely within our laboratory for oat and barley samples.

**EVALUATION:**

The Streamlined β-glucan method has been successfully evaluated by AOAC International (Method 995.16), AACC (Method 32-23.01) and ICC (Method No. 166).

**SPECIFICITY:**

The assay is specific for mixed-linkage [(1-3)(1-4)]-β-D-glucan.

**SAFETY:**

Safety data sheets are available for all products at www.megazyme.com.

**KITS:**

Kits suitable for performing 100 assays are available from Neogen.

| | |
|---|---|
| **Bottle 1:** | Lichenase [specific, *endo*-(1-3)(1-4)-β-D-glucan 4-glucanohydrolase] suspension (1 mL).<br>Store at 4°C. See individual label for expiry date. |
| **Bottle 2:** | β-Glucosidase (1 mL) suspension.<br>Store at 4°C. See individual label for expiry date. |
| **Bottle 3:** | **GOPOD Reagent Buffer.** Buffer (50 mL, pH 7.4), *p*-hydroxybenzoic acid and sodium azide (0.09% w/v).<br>Store at 4°C. See individual label for expiry date. |

**Bottle 4:** **GOPOD Reagent Enzymes.** Glucose oxidase plus peroxidase and 4-aminoantipyrine. Freeze-dried powder.
Store below -10°C. See individual label for expiry date.

**Bottle 5:** D-Glucose standard solution (5 mL, 1.0 mg/mL) in 0.2% (w/v) benzoic acid.
Store sealed at room temperature. See individual label for expiry date.

**Bottle 6:** Standardised barley flour control. β-Glucan content shown on vial label.
Store sealed at room temperature. See individual label for expiry date.

**Bottle 7:** Standardised oat flour control. β-Glucan content shown on vial label.
Store sealed at room temperature. See individual label for expiry date.

**PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:**

**1.** Dilute the contents of **bottle 1** (lichenase) to 20.0 mL with 20 mM sodium phosphate buffer (pH 6.5). This is **solution 1** (**diluted lichenase**). Divide into appropriately sized aliquots and store in polypropylene tubes below -10°C between use and store on ice during use.
Stable for ≥ 2 years below -10°C.

**NOTE:** It is imperative that the lichenase is not cross contaminated with β-glucosidase.

**2.** Dilute the entire contents of **bottle 2** (β-glucosidase) to 20.0 mL with 50 mM sodium acetate buffer (pH 4.0). This is **solution 2** (**diluted β-glucosidase**). Divide into appropriately sized aliquots and store in polypropylene tubes below -10°C between use and store on ice during use.
Stable for ≥ 2 years below -10°C.

**3.** Dilute the contents of the **GOPOD Reagent Buffer** bottle to 1 L with distilled water (this is **solution 3**). Use immediately.

**NOTE:**
1. On storage, salt crystals may form in the concentrated buffer. These must be completely dissolved when this buffer is diluted to 1 L with distilled water.
2. This buffer contains 0.09% (w/v) sodium azide.
This is a poisonous chemical and should be treated accordingly.

**4.** Dissolve the contents of **GOPOD Reagent Enzyme** bottle in 20 mL of **solution 3** and quantitatively transfer this to the bottle containing the remainder of **solution 3**. Cover this bottle with aluminium foil to protect the enclosed reagent from light. This is **Glucose Determination Reagent** (**GOPOD Reagent**).
Stable for ≥ 1 month at 4°C or ≥ 12 months below -10°C.

If this reagent is to be stored in the frozen state, preferably it should be divided into aliquots. Do not freeze/thaw more than once.

When the reagent is freshly prepared it may be light yellow or light pink in colour. The solution may develop a stronger pink colour upon storage at 4°C.

The absorbance of this solution should be less than 0.05 when read against distilled water.

**BUFFERS (NOT SUPPLIED):**

1. **20 mM Sodium phosphate buffer, pH 6.5.**
   Dissolve 3.12 g of sodium phosphate monobasic dihydrate ($NaH_2PO_4.2H_2O$) in 900 mL of distilled water and adjust the pH to 6.5 by the addition of 100 mM sodium hydroxide (4 g/L) (approx. 50 mL is required). Adjust the volume to 1 L. Add 0.2 g of sodium azide.
2. **50 mM Sodium acetate buffer, pH 4.0.**
   Add 2.9 mL of glacial acetic acid to 900 mL of distilled water. Adjust to pH 4.0 by the addition of 1 M sodium hydroxide solution. Adjust the volume to 1 L.
3. **200 mM Sodium acetate buffer, pH 4.0.**
   Add 11.6 mL of glacial acetic acid to 900 mL of distilled water. Adjust to pH 4.0 by the addition of 1 M sodium hydroxide solution. Adjust the volume to 1 L.

**REAGENTS (NOT SUPPLIED):**

Read the assay protocol carefully to determine the reagents required for the appropriate method (A/B/C).

1. **50 % Ethanol (v/v).**
   Add 500 mL of ethanol (100% v/v) to 500 mL of distilled water. Store in a 1 L Duran bottle.
2. **95% Ethanol (v/v).**
   95% Ethanol (v/v) reagent grade or similar

**EQUIPMENT (RECOMMENDED):**

1. Polypropylene tubes/containers with caps (35 mL capacity).
2. Glass test-tubes (12 mL capacity) [Plastic test tubes of the same dimension are also suitable for use].
3. Micro-pipettors, e.g. Gilson® Pipetman® (100 µL and 200 µL).
4. Positive displacement pipettor, e.g. Eppendorf® Multipette®
   - with 5.0 mL Combitip® (to dispense 0.1 mL aliquots of buffer and buffered β-glucosidase solution).
5. Adjustable-volume dispensers
   0-5.0 mL (for phosphate buffer).
   3.0 mL (for glucose oxidase/peroxidase reagent).
   0-25.0 mL (for distilled water).

6. Laboratory oven.
7. Analytical and top-pan balances.
8. Spectrophotometer set at 510 nm (see point 1 under Useful Hints, page 9).
9. Vortex mixer.
10. Thermostated water bath set at 50°C (or 40°C for the original version of the method, page 10).
11. Stopwatch.
12. Whatman® No. 41 filter circles.
13. Centrifuge (in conjunction with preparation of malt, wort and beer).
14. Laboratory mill with 0.5 mm screen.
15. Boiling water bath.

**CONTROLS AND PRECAUTIONS:**

1. With each set of determinations, reagent blanks and D-glucose standards of 100 μg should be included, in duplicate.

   The **reagent blank** comprises 0.1 mL distilled water + 0.1 mL 50 mM sodium acetate buffer pH 4.0 + 3.0 mL of **GOPOD Reagent.**

   The **glucose standard** comprises 0.1 mL 50 mM sodium acetate buffer pH 4.0 + 0.1 mL D-glucose standard (100 μg/0.1 mL) + 3.0 mL **GOPOD Reagent.**
2. With each set of determinations at least one standardised barley flour control should also be included.
3. It is imperative that the lichenase enzyme preparation is **not** cross-contaminated with the β-glucosidase preparation (the reverse is not a problem).

**(A) ASSAY PROCEDURE FOR OAT AND BARLEY FLOUR AND FIBER SAMPLES - STREAMLINED METHOD (AOAC Method 995.16, AACC Method 32-23 and ICC Standard Method No. 166).**

This procedure is ideal for all dry samples particularly those containing high levels of β-glucan (e.g. processed oat bran products).

**METHOD:**

1. Mill barley, oats or fiber sample (approx. 50 g) to pass a 0.5 mm screen using a centrifugal mill.
2. Add flour sample (80-120 mg; weighed accurately) to a glass centrifuge tube

(16 x 120 mm; 17 mL capacity). Tap the tube to ensure that all sample falls to the bottom of the tube.

3. Wet the sample with 0.2 mL of aqueous ethanol (50% v/v) to aid dispersion. Add 4.0 mL of 20 mM sodium phosphate buffer pH 6.5 and stir the contents on a vortex mixer.
4. On mixing, immediately place the tube in a boiling water bath and incubate for 60 sec. Vigorously stir the mixture on a vortex mixer, incubate at 100°C for a further 2 min and stir again.
5. Incubate the tube plus contents at 50°C and allow to equilibrate for 5 min.
6. Add 0.2 mL of **solution 1** (**diluted lichenase**) and stir the tube contents. Seal the tube with parafilm and incubate for 1 h at 50°C, with regular vigorous stirring (i.e. 3-4 times) on a vortex mixer. Continuous stirring is recommended where possible.
7. Add 5.0 mL of 200 mM sodium acetate buffer pH 4.0 and vigorously mix the tube contents on a vortex mixer.
8. Allow the tube to equilibrate to room temperature (5 min) and centrifuge (1,000 *g*, 10 min). Carefully and accurately dispense aliquots (0.1 mL) into the bottom of three test tubes (12 mL capacity).
9. Add 0.1 mL of **solution 2** (**diluted β-glucosidase**) to two of these tubes (the reaction). To the third (the reaction blank), add 0.1 mL of 50 mM acetate buffer pH 4.0. Incubate all tubes at 50°C for 10 min.
10. Add 3.0 mL **GOPOD Reagent** to each tube and incubate at 50°C for a further 20 min.
11. Remove the tubes from the water bath and measure the absorbance at 510 nm against reagent blank within 1 h.

**NOTE:** The amount of D-glucose present in the test tube (i.e. in the 0.1 mL of sample being analysed) should range between 4 and 100 µg. The sample solution before the β-glucosidase treatment must be diluted sufficiently with 200 mM sodium acetate buffer (pH 4.0) to yield a sugar concentration between 0.04 and 1.0 g/L, which is equivalent to approx. 0.35 and 8.5% of β-glucan in the original sample. For example, if a sample contains 20% of beta-glucan it should be diluted 3-fold with 200 mM sodium acetate buffer (pH 4.0) before dispensing aliquots for incubation with β-glucosidase.

Alternatively for samples containing high β-glucan, e.g. Oatwell (> 50% β-Glucan), the sample size should be reduced to 50 mg and the volume should be adjusted to 100 mL with 200 mM sodium acetate buffer (pH 4.0) after lichenase treatment.

**(B) ASSAY PROCEDURE FOR COOKED, TOASTED OR EXTRUDED CEREAL PRODUCTS – STREAMLINED METHOD (AOAC Method 995.16, AACC Method 32-23 and ICC Standard Method No. 166).**

In the analysis of β-glucan in cooked, toasted or extruded cereal products, the sample should be pre-extracted with aqueous ethanol to remove free sugars and to reduce the levels of fats and oils.

**METHOD:**

1. Mill food product (approx. 50 g) to pass a 0.5 mm screen using a centrifugal mill.
2. Add sample (~ 200 mg; weighed accurately) to a glass centrifuge tube (16 x 120 mm; 17 mL capacity). Tap the tube to ensure that all sample falls to the bottom of the tube.
3. Add 5.0 mL of aqueous ethanol (50% v/v) and incubate the tubes in a boiling water bath for 5 min. Mix the contents on a vortex stirrer and add a further 5.0 mL of 50% (v/v) aqueous ethanol. Mix.
4. Centrifuge the tubes for 10 min at 1,800 *g* (approx. 3,000 rpm). Discard the supernatant.
5. Resuspend the pellet in 5.0 mL of 50% (v/v) aqueous ethanol and stir on the vortex mixer. Add a further 5.0 mL of 50% aqueous ethanol. Stir on the vortex mixer, centrifuge and discard the supernatant (as in step 4).
6. Suspend the pellet in 4.0 mL of 20 mM sodium phosphate buffer pH 6.5 and incubate the tube at 50°C for 5 min.
7. Add 0.2 mL of **solution 1** (**diluted lichenase**) and stir the tube contents. Seal the tube with parafilm and incubate for 1 h at 50°C, with regular vigorous stirring (i.e. 3-4 times) on a vortex mixer. Continuous stirring is recommended where possible.
8. Add 2.0 mL of 200 mM sodium acetate buffer pH 4.0 and vigorously mix the tube contents on a vortex mixer.
9. Proceed from **Step 8 of method (A).**

**(C) ASSAY PROCEDURE FOR MILKSHAKE, YOGURT AND OTHER LIQUID SAMPLES (ALCOHOL PRECIPITATION).**

1. Weigh accurately a glass centrifuge tube (16 x 120 mm; 17 mL capacity).
2. Add 3 mL of sample to the tube and heat in a boiling water bath for 5 min. Allow to cool to room temperature.
3. Add 3 mL of 95% aqueous ethanol to the tube and mix the contents on a vortex stirrer. Add a further 5.0 mL of 95% aqueous ethanol. Mix well on a vortex stirrer.

4. Centrifuge the tubes for 10 min at 1,800 *g* (approx. 3,000 rpm). Discard the supernatant.
5. Resuspend the pellet in 8.0 mL of 50% (v/v) aqueous ethanol and stir on the vortex mixer. Centrifuge and discard the supernatant (as in step 4).
6. Suspend the pellet in 20 mM sodium phosphate buffer pH 6.5 adjusting the volume to 4.0 mL (by weight), from the known weight of the empty tube. Incubate the tube at 50°C for 5 min.
7. Proceed from **Step 6 of method (A).**

**NOTE:** If the absorbance values received for a sample exceed the absorbance values obtained for glucose standard the samples must be diluted with 200 mM sodium acetate buffer (pH 4.0) to bring them on scale before dispensing aliquots for incubation with β-glucosidase.

**CALCULATIONS:**

**For Solid samples:**

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

$$\beta\text{-glucan (\% w/w) ("as is")} = \Delta A \times F \times \frac{FV}{0.1} \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180} \times D$$

$$= \Delta A \times \frac{F}{W} \times FV \times D \times 0.9$$

**where:**

$\Delta A$ = absorbance after β-glucosidase treatment (reaction) minus reaction blank absorbance.

F = factor for the conversion of absorbance values to μg of glucose.

$$= \frac{100\ (\mu g \text{ of D-glucose})}{\text{absorbance of } 100\ \mu g \text{ of D-glucose}}$$

FV = final volume (i.e. equals 9.4 mL for oat and barley flour in method (A); 6.4 mL for cooked, toasted and extruded cereal products in example (B); 100 mL for samples containing > 50% β-glucan, see note on page 6).

0.1 = volume of sample analysed.

$\frac{1}{1000}$ = conversion from µg to mg.

$\frac{100}{W}$ = factor to express β-glucan content as a percentage of sample weight.

W = the weight in mg ("as is" basis) of the sample analysed.

$\frac{162}{180}$ = factor to convert from free D-glucose, as determined, to anhydro-D-glucose, as occurs in β-glucan.

D = further dilution prior to incubation with β-glucosidase (if required).

**β-glucan % w/w (dry wt. basis)**:

$$= \textbf{β-glucan \% w/w (as is)} \times \frac{100}{100 - \text{moisture content (\% w/w)}}$$

**For (Liquid samples; g/100 mL):**

**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).

$$\textbf{β-glucan (g/100 mL)} = \Delta A \times F \times \frac{9.2}{3.0} \times 1000 \times \frac{1}{1000} \times \frac{1}{1000} \times \frac{162}{180} \times D$$

$$= \Delta A \times F \times D \times 0.00276$$

**where:**

ΔA = absorbance after β-glucosidase treatment (reaction) minus reaction blank absorbance.

F = factor for the conversion of absorbance values to µg of glucose.

$$= \frac{100\ (\mu g \text{ of D-glucose})}{\text{absorbance of } 100\ \mu g \text{ of D-Glucose}}$$

$\frac{9.2}{3.0}$ = volume correction factor: 3.0 mL aliquots of sample were treated with ethanol and volume was readjusted to 9.2 mL) (i.e. 4.0 mL + 0.2 mL **solution 1 (diluted lichenase)** + 5.0 mL sodium acetate buffer).

1000 = volume adjustment factor (0.1 mL was analysed but results are presented per 100 mL of sample

$\frac{1}{1000}$ = conversion from µg to mg.

$\frac{162}{180}$ = factor to convert from free D-glucose, as determined, to anhydro-D-glucose, as occurs in β-glucan.

D = further dilution prior to incubation with β-glucosidase (if required).

**(D) ASSAY PROCEDURE FOR BARLEY APPROVED BY EUROPEAN BREWING CONVENTION**:

**USEFUL HINTS:**

1. After incubation of samples with **solution 1** (**diluted lichenase**) it is suggested that the volume of the reaction mixture* be adjusted to 30.0 mL by the addition of 24.0 mL of distilled water via a dispenser.

* Assume the volume to be 6.0 mL; approx. 0.2 mL is lost during the heating step.

2. In step 5 of the assay procedure, if the solution becomes very viscous after the 5 min boiling step, add 5.0 mL of distilled water and stir well on a vortex mixer. After reaction with **solution 1** (**diluted lichenase**) adjust the volume to 30.0 mL by the addition of 19.0 mL of distilled water.

**NOTE:** If the solution is very viscous there may be some problem with the diffusion of lichenase. Adding 5.0 mL of distilled water will alleviate this problem.

3. If glass, rather than polypropylene, tubes are used in step 5 of the assay procedure, reduce the time of incubation in the boiling water bath to 45 sec initially, vortex the contents and incubate for a further 45 sec in the boiling water bath (i.e. total of 1.5 min).

**METHOD:**

1. Mill barley to pass a 0.5 mm screen using a Tecator Cyclotec® mill (uniform, fine milling is essential).
2. Accurately weigh barley flour samples (approx. 0.5 g) of known moisture content* into polypropylene tubes (refer to Equipment, point 1, page 3).

* See footnote under Example Results Sheet on page 10.

3. Add an aliquot (1.0 mL) of aqueous ethanol (50% v/v) to each tube to aid in the subsequent dispersion of samples.
4. Add 5.0 mL of 20 mM sodium phosphate buffer pH 6.5 and stir the tubes on a vortex mixer.
5. Incubate the tubes in a boiling water bath for approx. 2 min (see point 2 and 3 under Useful Hints). Remove the tubes and vigorously stir them on a vortex mixer. Heat the tubes for a further 3 min in the boiling water bath (mixing after 2 min prevents formation of a lump of gel material
6. Cool the tubes to 40°C and add 0.2 mL of **solution 1** (**diluted lichenase**) to each tube. Cap the tubes, stir and incubate at 40°C for 1 h.
7. Adjust the volume in each tube to 30.0 mL by the addition of distilled water (see point 1 under Useful Hints, page 9).
8. Thoroughly mix the contents of the tubes and filter an aliquot from each tube through a Whatman No. 41 filter circle (or centrifuge an aliquot at approx. 1,000 *g* for 10 min).
9. Carefully and accurately transfer aliquots (0.1 mL) from each filtrate to the bottom of three test tubes.
10. Add 0.1 mL of 50 mM sodium acetate buffer pH 4.0 to one of these (the reaction blank), while to the other two (the reaction) add 0.1 mL of **solution 2** (**diluted β-glucosidase**). Incubate the tubes at 40°C for 15 min.
11. Add **GOPOD Reagent** (3.0 mL) to each tube and incubate at 40°C for 20 min (see point 3 under Controls and Precautions, page 4).
12. Measure the absorbance at 510 nm for each reaction (EA) and reaction blank ($E_{BI}$).

**NOTE:** With the **GOPOD Reagent** employed in this kit, the colour complex which is formed is stable at room temperature. The absorbance should be measured within 2 h.

**EXAMPLE RESULTS SHEET:**

| Sample | Sample weight (mg) | | Absorbances (510 nm) | | | β-Glucan |
|---|---|---|---|---|---|---|
| | Fresh | Dry (corrected) | $E_{BI}$ | $E_A$ | ΔA | % (w/w) |
| e.g. Clipper | 495 | 420 | 0.012 | 0.460<br>0.455 | 0.448<br>0.443 | 2.86<br>2.83 |

$$\text{Dry weight} = \text{fresh weight} \times \frac{100 - \text{moisture content (\%)}^*}{100}$$

* In general this is determined by NIR reflectance. Alternatively, this can be determined by observing weight loss on storage of flour samples (0.5 g) at 80° C for 20 h. The moisture content of cereal flour samples is consistently in the range of 10-14%.

**(E) ASSAY PROCEDURE FOR MALT, SPENT GRAIN, BEER AND WORT APPROVED BY EUROPEAN BREWING CONVENTION**

**Malt**:

1. To 1.0 g of malt flour (milled to pass a 0.5 mm screen) or lyophilised barley samples removed during the malting process, add 5.0 mL of aqueous ethanol (50% v/v).
2. Incubate in a boiling water bath for 5 min. Mix the contents on a vortex stirrer and add a further 5.0 mL of 50% (v/v) aqueous ethanol. Mix.
3. Centrifuge for 10 min at 1,000 *g*. Discard the supernatant.
4. Resuspend the pellet in 10.0 mL of 50% (v/v) aqueous ethanol, centrifuge and discard the supernatant (as in step 3. above).
5. Suspend the pellet in 5.0 mL of 20 mM sodium phosphate buffer pH 6.5.
6. Assay for β-glucan as per the Assay Procedure for **method D** (barley) from step 5.

**Spent Grain:**

Either wash spent grain with hot water (approx. 75°C), and then lyophilise, or lyophilise without washing. Mill this material to pass a 0.5 mm screen and analyse for β-glucan content and perform calculations by the same procedure as employed for the malt samples.

**Beer or Wort:**

1. Degas beer by heating an aliquot to approx. 80°C in a boiling water bath. Allow to cool.
2. To 5.0 mL of wort or degassed beer in a pre-weighed centrifuge tube (12 mL capacity), add 2.5 g of finely milled ammonium sulphate crystals.
3. Seal the tube with Parafilm® and dissolve the ammonium sulphate by careful inversion (to avoid frothing).
4. Allow the tube to stand for approx. 20 h at 4°C.
5. Centrifuge at 1,000 *g* for 10 min on a bench centrifuge.
6. Discard the supernatant.
7. Resuspend the pellet by thoroughly vortexing with 1.0 mL of 50% (v/v) aqueous

ethanol. Add a further 10.0 mL of 50% (v/v) aqueous ethanol and mix well by inversion of the tube.

8. Centrifuge at 1,000 *g* for 5 min. Discard the supernatant.
9. Repeat the ethanol-washing procedure by resuspending the pellet etc. as in steps 7 and 8 above.
10. Discard the supernatant.
11. Resuspend the pellet in 20 mM sodium phosphate buffer pH 6.5: for wort, adjust the volume to 4.8 mL (by weight), for beer, adjust the volume to 1.8 mL (by weight).
12. Add 0.2 mL **solution 1** (**diluted lichenase**) and incubate at 40°C for 5 min. Centrifuge at 1,000 *g* for 10 min then proceed as per the assay procedure for **method D** (barley) starting from step 9.

**NOTE:** For wort samples containing low levels of β-glucan, incubate a larger aliquot of sample solution (up to 0.5 mL) with **solution 2** (**diluted β-glucosidase**). Use this larger aliquot size also for the blank. The D-glucose standard must also be adjusted accordingly with distilled water. Modify calculations accordingly.

**CALCULATIONS:**

For barley, malt and spent grain

$$\textbf{β-glucan (\% w/w)} = \Delta A \times F \times 300 \times \frac{1}{1000} \times \frac{100}{W} \times \frac{162}{180}$$

$$= \Delta A \times \frac{F}{W} \times 27$$

For wort

$$\textbf{β-glucan (mg/L)} = \Delta A \times F \times 10{,}000 \times \frac{1}{1000} \times \frac{5}{5} \times \frac{162}{180}$$

$$= \Delta A \times F \times 9$$

For beer

$$\textbf{β-glucan (mg/L)} = \Delta A \times F \times 10{,}000 \times \frac{1}{1000} \times \frac{2}{5} \times \frac{162}{180}$$

$$= \Delta A \times F \times 3.6$$

**Where:**

$\Delta A$ = absorbance after β-glucosidase treatment (reaction) minus reaction blank absorbance.

F = factor for the conversion of absorbance values to µg of glucose.

$$= \frac{\text{100 (µg of D-glucose)}}{\text{absorbance of 100 µg of D-glucose}}$$

300 = volume correction (i.e. 0.1 mL taken from 30.0 mL).

10,000 = volume adjustment factor (0.1 mL was analysed but results are presented per litre of sample).

$\frac{1}{1000}$ = conversion from µg to mg.

$\frac{1}{1000}$ = conversion from µg to mg.

W = the calculated dry weight of the sample analysed, in mg (refer to example results sheet

$\frac{5}{5}$ = volume correction factor. For wort samples, 5.0 mL aliquots were treated with precipitant (ammonium sulphate) and the volume was readjusted to 5.0 mL (i.e. 4.8 mL + 0.2 mL **solution 1** (**diluted lichenase**).

$\frac{2}{5}$ = volume correction factor. For beer samples, 5.0 mL treated with treated with precipitant (ammonium sulphate) and the volume was readjusted to 2.0 mL (i.e. 1.8 mL + 0.2 mL **solution 1** (**diluted lichenase**).

$\frac{162}{180}$ = factor to convert from free D-glucose, as determined, to anhydro-D-glucose, as occurs in β-glucan.

**COMPARISON OF METHODS:**

The streamlined β-glucan method **(A)** has been compared to AACC Method 32-22 (the AACC modification of the original 'Megazyme' method)[1] in an interlaboratory evaluation and the results obtained with both methods were very similar. Results with the "Streamlined Method" **(method A)** are shown in Table 1. With this method, more than 100 samples can be analysed by a single analyst in one day. This compares to about 20 samples with AACC Method 32-22.

***Table 1:*** Method Performance for Determination of β-D-Glucan in Oats by Streamlined Enzymatic Method **(Method A).**[a]

| Sample | Mean, % dry basis | $S_r$ | $S_R$ | $RSD_r$ % | $RSD_R$ % | $r^b$ | $R^c$ |
|---|---|---|---|---|---|---|---|
| Oat flour | 2.73 | 0.083 | 0.241 | 3.1 | 8.8 | 0.232 | 0.675 |
| Oat bran | 6.39 | 0.296 | 0.456 | 4.6 | 7.1 | 0.829 | 1.277 |
| Rolled oats | 4.27 | 0.283 | 0.315 | 6.6 | 7.4 | 0.792 | 0.882 |
| Oat bran (breakfast cereal) | 3.93 | 0.484 | 0.484 | 12.3 | 12.3 | 1.355 | 1.355 |
| Instant oat bran | 8.00 | 0.480 | 0.524 | 6.0 | 6.6 | 1.344 | 1.467 |

a Based on results from 8 laboratories; no outliers identified

b $r = 2.8 \times s_r$

c $R = 2.8 \times s_R$

**STANDARDISATION OF ENZYME ACTIVITY:**

**β-Glucosidase** was standardised using *p*-nitrophenyl β-glucoside as substrate. One unit is defined as the amount of enzyme required to release one µmole of *p*-nitrophenol from *p*-nitrophenyl β-glucoside (10 mM) per min at pH 4.0 and 40°C. **Lichenase** activity was determined on barley β-glucan (10 mg/mL) in sodium phosphate buffer (pH 6.5) at 40°C using the Nelson/Somogyi reducing sugar procedure.[1] One Unit of activity is defined as the amount of enzyme required to release one µmole of D-glucose reducing-sugar equivalents per min under the defined assay conditions.

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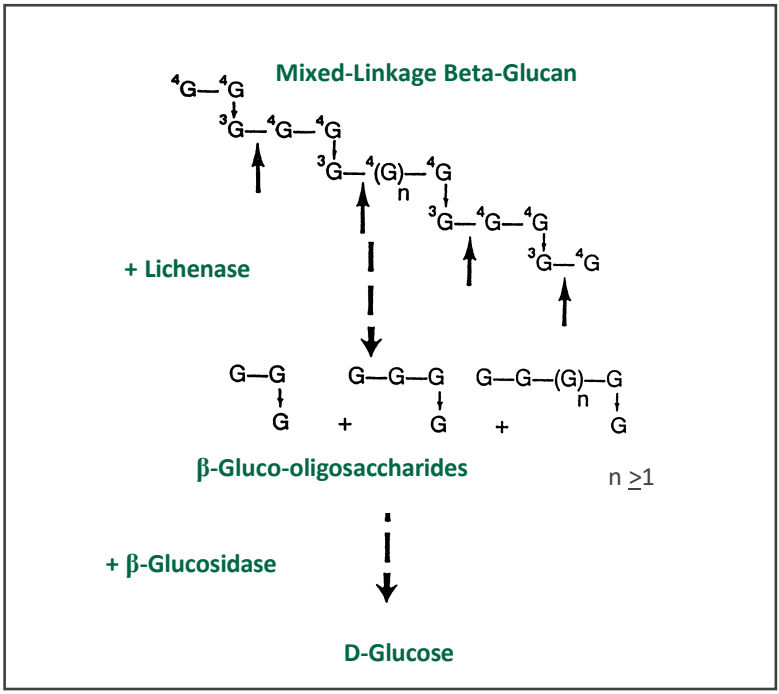


***Scheme 1.*** Principle of the mixed-linkage beta-glucan assay procedure.

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