



CALCOFLUOR / FIA : STANDARD BARLEY β -GLUCAN

08/2002

Availability:

This β -glucan standard is supplied as a freeze dried powder in the presence of a bulking agent. The contents of one 25 ml vial (40 mg of β -glucan) is dissolved in 20 ml of water and then diluted to give a β -glucan concentration of 400 μ g/ml (by a Megazyme Enzymic method; overpage).

Preparation of standard:

Add 20 ml of distilled or demineralised water to a vial containing standard β -glucan (40 mg plus bulking agent). Add a magnetic stirrer bar (5 x 15 mm) and stir the contents on a magnetic stirrer hot-plate. Set the hot plate temperature to about 150°C and continue stirring until the β -glucan completely dissolves and the solution becomes crystal clear. (This occurs after 2-5 min, when the temperature of the vial reaches 65-70°C). Quantitatively transfer the contents of the vial to a 100 ml volumetric flask, using a small funnel in the flask and a water wash bottle to ensure complete transfer of all of the β -glucan. Adjust to volume and mix well by inversion.

Transfer the solution to a clean and dry 100 ml Pyrex bottle (with tight fitting plastic lid). If the solution is to be used over an extended period of time, either add 20 mg of sodium azide or two drops of toluene as a preservative. This solution must be stored at room temperature to minimise the possibility of self-association and precipitation of the β -glucan. In our experience, with this β -glucan preparation, there was no evidence of precipitation over a 3 month period on storage at room temperature. This was determined by centrifuging an aliquot of the solution at 12,000 g for 10 min, and analysing the β -glucan concentration in the supernatant solution obtained (i.e. there was no evidence for any precipitate formation).

The concentration of β -glucan in the standard solution can be checked using a mixture of pure β -glucosidase and cellulase (Megazyme) as described on the next page. In the Megazyme β -glucan test kit for the measurement of β -glucan in barley, malt, wort and beer (EBC Methods 3.11.1, 4.16.1 & 8.11.1) the enzymes lichenase and β -glucosidase are used. Lichenase is used (instead of cellulase) because the lichenase hydrolyses only barley β -glucan with no action on cellulose (which also occurs in milled barley). Lichenase has a pH optima of 6.5, whereas that for β -glucosidase it is pH 4.0. In analysing pure barley β -glucan (as in the FIA β -glucan standard) there are no other β -glucans present (e.g. amorphous cellulose), thus, it is much more convenient to use a mixture of pure β -glucosidase plus cellulase, both of which have optimal activity at pH 4.0.

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Enzymic determination of β -glucan in the standard solution:

NOTE: This is performed only to confirm the concentration of the β -glucan. It is not a necessary part of using the β -glucan solution as an FIA/Calcofluor standard.

1. Enzyme Mixture:

Dilute 1 vial of Megazyme β -glucosidase (cat. no. E-BGLUC; 120 Units) to 40 ml in 100 mM sodium acetate buffer (pH 4.0). Add 0.4 ml of Megazyme cellulase (E-CELTR; 1,000 U/ml, i.e. 400 Units) and mix well.

Divide the solution into 5-10 ml aliquots and store in polypropylene tubes at -20°C between use. The enzyme mixture is stable to repeated freeze-thaw cycles.

2. Megazyme Glucose Test Kit (K-GLUC).

3. Assay Procedure:

a. Carefully dispense 0.2 ml of the β -glucan solution (400 $\mu\text{g}/\text{ml}$) directly to the bottoms of four glass test-tubes (16 x 100 mm).

b. Add 0.1 ml of the β -glucosidase/cellulase mixture, mix well and incubate at 50°C for 15 min.

c. Add 3.0 ml of GOPOD Reagent (Megazyme Glucose Assay Kit) and incubate at 50°C for 20 min along with **reaction blanks** and **glucose standards**.

- **Reaction Blank:** (duplicate) 0.1 ml of water + 0.2 ml of 100 mM sodium acetate buffer (pH 4.0) + 3.0 ml of GOPOD Reagent.

- **Glucose Standard** (quadruplicate): 0.1 ml of glucose standard (1 mg/ml) + 0.2 ml of 100 mM sodium acetate buffer (pH 4.0) + 3.0 ml of GOPOD Reagent.

d. After incubation, read the absorbance of samples and glucose standards against the reaction blank at 510 nm and calculate the β -glucan concentration as follows:

Beta-Glucan Concentration ($\mu\text{g}/\text{ml}$);

$$= \Delta E \times F \times \frac{162}{180} \times 5 = \Delta E \times F \times 4.5$$

where:

ΔE = Absorbance (reaction) - Absorbance (blank).

F = $\frac{100 \text{ } (\mu\text{g of glucose})}{\text{absorbance for 100 } \mu\text{g of glucose}}$

$\frac{162}{180}$ = Adjustment from free glucose to anhydro-glucose (as occurs in β -glucan).

5 = conversion from 0.2 ml (as assayed) to 1.0 ml.