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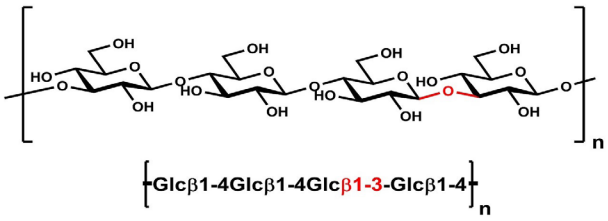
β-Glucan CFA Standard (Lot 200102)

P-BGCFA (β-glucan for continuous flow analyser) **03/23**

CAS: 9041-22-9
Source: Barley flour

STRUCTURE:



Schematic representation of the β-glucan polysaccharide contained in the vials.

DESCRIPTION:

This β-glucan standard is suitable for the measurement of β-glucan in barley, malt, wort and beer using fluorescent analysis in conjunction with Calcofluor as per EBC, ASBC and MEBAK official methods. It is supplied as a freeze dried powder in the presence of a bulking agent.

PREPARATION OF STANDARD:

Add 20 mL of distilled or demineralised water to a vial containing standard β-glucan (~ 35 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 approx. 150°C and continue stirring until the β-glucan completly dissolves and the solution becomes 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 graduated cylinder, using a water wash bottle to ensure complete transfer of all of the β-glucan. Adjust to the volume provided on the vial label and mix well by inversion to give a β-glucan concentration of 400 µg/mL (the concentration can be checked using the enzymic method; next page).

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, add 20 mg of sodium azide 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 is no evidence of precipitation over a 3 month period on storage at room temperature.

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.

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 CFA/Calcofluor standard.*

1. Enzyme Mixture:
Dilute 120 Units of β-glucosidase (cat. no. **E-BGLUC**, i.e. 3 mL at 40 U/mL) to 40 mL in 100 mM sodium acetate buffer (pH 4.0). Add 400 Units of cellulase (**cat. no. E-CELAN**, i.e. 0.4 mL at 1000 U/mL) and mix well. *Divide the solution into 5-10 mL aliquots and store in polypropylene tubes below -10°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 µg/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 (cat. no. **K-GLUC**) 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 (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 (µg/mL);

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

where:

ΔA = Absorbance (reaction) - Absorbance (blank)

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

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

5 = conversion from 0.2 mL (as assayed) to 1.0 mL