



13/6/2009

**Suggested Modifications of the Standard β -Glucan Procedure for
Liquid Samples and Samples High in β -Glucan**

- A. Measurement of β -Glucan in High β -Glucan Containing Samples
e.g. Oatwell (~ 50% β -Glucan)**
1. Weigh approx. 50 mg of sample into a 16 x 120 mm glass test tube.
 2. Add 0.2 mL of aqueous ethanol (50 % v/v).
 3. Add 5 mL of 20 mM sodium phosphate buffer (pH 6.5), stir the tube on a vortex mixer and incubate at $\sim 100^{\circ}\text{C}$ for 2 min. Mix again on a vortex mixer and incubate at $\sim 100^{\circ}\text{C}$ for a further 4 min. Cool to 50°C .
 4. Add 0.2 mL of lichenase (100 U/mL) and incubate with continual stirring (e.g. using the Megazyme MultiStir bath) at 50°C for 1 h.
 5. Adjust the volume to 100 mL with 100 mM sodium acetate buffer (pH 4.0).
 6. If necessary, filter an aliquot of each solution through Whatman No. 1 (9 cm) filter circles, or centrifuge at 1,000 g for 10 min.
 7. Remove 0.1 mL aliquots (in triplicate) and incubate two of these with 0.1 mL of β -glucosidase (2 U/mL) for 15 min. To the third, add 0.1 mL of 100 mM sodium acetate buffer (pH 4.0) (the sample blank).
 8. Add 3.0 mL of GOPOD Reagent and incubate at 50°C for 20 min. Measure the absorbance at 510 nm against the reagent blank (refer to booklet). Run control glucose solution (in quadruplicate) concurrently.

Alternatively, use method B described below. In our hands, similar values were obtained with both formats.

B. Measurement of β -Glucan in Very High β -Glucan Containing Samples e.g. Glucagel (> 80% β -Glucan)

(NOTE: This method cannot be used for samples containing free glucose as the cellulase employed releases some glucose from the β -glucan during hydrolysis).

1. Weigh approx. 50 mg of sample into a 16 x 120 mm glass test tube.
2. Add 0.2 mL of aqueous ethanol (50 % v/v).
3. Add 5 mL of 20 mM sodium acetate buffer (pH 4.5), stir the tube on a vortex mixer and incubate at $\sim 100^\circ\text{C}$ for 2 min. Mix again on a vortex mixer and incubate at $\sim 100^\circ\text{C}$ for a further 4 min. Cool to 50°C .
4. Add 0.2 mL of cellulase (100 U/mL)(Megazyme E-CELTR) and incubate with continual stirring (e.g. using the Megazyme MultiStir bath) at 50°C for 1 h.
5. Adjust the volume to 100 mL with 100 mM sodium acetate buffer (pH 4.0).
6. If necessary, filter an aliquot of each solution through Whatman No. 1 (9 cm) filter circles, or centrifuge at 1,000 g for 10 min.
7. Remove 0.1 mL aliquots (in duplicate) and incubate them with 0.1 mL of β -glucosidase (2 U/mL) for 15 min.
8. Add 3.0 mL of GOPOD Reagent and incubate at 50°C for 20 min. Measure the absorbance at 510 nm against the reagent blank (refer to booklet). Un control glucose solution (in quadruplicate) concurrently.

C Measurement of β -Glucan in MilkShake, Yogurt and other Liquid Products.

a. Alcohol Precipitation

1. Add 3 mL of solution to a pre-weighed glass test-tube (16 x 120 mm) and heat in a boiling water bath for 5 min. Allow to cool to room temperature.
2. Add 9 mL of ethanol (95 % v/v) and stir the tube vigorously on a vortex mixer. Leave the tube at room temperature for 5 min and then centrifuge at 1,000 g for 10 min.
2. Suspend the pellet in 66% v/v aqueous ethanol. Re-centrifuge at 1,000 g for 10 min.
3. Dissolve the pellet in 2 mL of sodium phosphate buffer (20 mM, pH 6.5) and adjust the weight of the sample to 4 g (from the known weight of the empty tube).

4. Add 0.2 mL of lichenase (50 U/mL) and incubate at 50°C for 1 h.
5. Proceed as for the standard β -glucan assay procedure for wort.

b. Borohydride Reduction

1. Add 2 mL of solution to a pre-weighed glass test-tube (16 x 120 mm) and heat in a boiling water bath for 5 min. Dilute to 10 mL with distilled water.
2. To 0.2 mL of this diluted sample solution, add 0.2 mL of sodium borohydride solution (10 mg/mL in 50 mM sodium hydroxide) and incubate at 40°C for 30 min
3. To each tube, add 0.5 mL of 0.2 M acetic acid and mix vigorously. (This adjusts the pH to approximately 4.0).
4. Remove 0.1 mL aliquots and incubate with a mixture of 0.1 mL of a mixture of β -glucosidase (2 U/mL) plus cellulase (50 U/mL) in 0.1 M sodium acetate buffer (pH 4.0) for 10 min at 40°C.
5. Measure D-glucose in the reaction solution using GOPOD reagent, and measure the absorbance at 510 nm against the reagent blank.
6. Reagent blank is prepared as for the sample except that sample solution is replaced by 0.2 mL of 0.1 M sodium acetate buffer (pH 4.0).
7. Glucose standard is prepared by mixing 0.1 mL of sodium acetate buffer (100 mM, pH 4.0) and 0.1 mL of glucose standard solution (1 mg/mL).
8. In all cases, add 3.0 mL of GOPOD Reagent and incubate at 50°C for 20 min. Measure absorbance at 510 nM against the reagent blank.