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Modifications of the Standard β-Glucan Procedure for Liquid Samples and Samples High in β-Glucan using Cellulase (*endo*-1,4-β-D-glucanase) (*Trichoderma longibrachiatum*); E-CELTR

(A) 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 form 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 ~100°C for 2 min. Mix again on a vortex mixer and incubate at ~100°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, Megazyme G-IBMKIII) 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; dilute a suitable aliquot of E-BGLUC 20-fold in 100 mM sodium acetate buffer pH 4.0) 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. Run control glucose solution (in quadruplicate; 100 µg) concurrently (Refer to K-BGLU booklet; "Controls and precautions" section, point 1.).

(B) Measurement of β-Glucan in MilkShake, Yogurt and other Liquid Products-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 200 mM acetic acid and mix vigorously. (This adjusts the pH to approx. 4.0).

4. Remove 0.1 mL aliquots and incubate with 0.1 mL of a mixture of β-glucosidase (2 U/mL; Megazyme E-BGLUC) plus cellulase (50 U/mL; Megazyme E-CELTR) in 0.1 M sodium acetate buffer (pH 4.0) for 15 min. at 40°C.

5. Add 3.0 mL of GOPOD Reagent and incubate at 40°C for 20 min. Measure the absorbance at 510 nm against the reagent blank. Run control glucose solution (in quadruplicate; 100 µg) concurrently (Refer to K-BGLU booklet; "Controls and precautions" section, point 1).