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Beta Glucan

DEFINITION

Beta Glucan is obtained by extraction from the cell wall of fermented and thermally processed Baker's yeast (*Saccharomyces cerevisiae*). It is comprised mainly of β-(1,3)/(1,6) branched glucan polymers. Small amounts of β-(1,6)-glucan and chitin are also expected to be present in the final product. It contains NLT 70% beta glucan, calculated as glucose after enzymatic hydrolysis, on the dried basis.

IDENTIFICATION

• **NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY (761).**

Standard solution: Dissolve 10 mg of [USP Beta Glucan RS](#) in 0.6 mL of dimethyl sulfoxide-*d*₆, and heat at 100° for 1 h. Then add 0.1 mL of D₂O, mix the solution, and transfer to an NMR tube.

Sample solution: Dissolve 10 mg of Beta Glucan in 0.6 mL of dimethyl sulfoxide-*d*₆, and heat at 100° for 1 h. Then add 0.1 mL of D₂O, mix the solution, and transfer to an NMR tube.

Analysis: Collect ¹H NMR spectra at 80°, and compare individual resonances from the *Sample solution* to those from the *Standard solution*. The major signals associated with Beta Glucan are shown in [Table 1](#).

Table 1

¹ H NMR Major Signals	USP Beta Glucan RS
H-1 (1,3)-glucan	4.52, d, J = 7.5 Hz, 1H
H-2, 4, and 5 (1,3-)	3.27–3.33, m, 3H
H-3 and 6b (1,3-)	3.45–3.48, m, 2H
H-6a (1,3-)	3.71, d, J = 11 Hz, 1H
H-1 (1,6)-glucan	4.27, d, J = 7.7 Hz, 1H

Integrate the area under the peaks five times for each sample, and average. Determine the relative percentage of (1,6) linked glucan in the portion of Beta Glucan taken:

Result = {A/(A + B)} × 100

A = integration values for the signal at 4.27 ppm, corresponding to H-1 from (1,6) glucan
B = integration values for the signal at 4.52 ppm corresponding to H-1 (1,3) glucan

Acceptance criteria: The ¹H spectrum of the *Sample solution* exhibits a chemical shift pattern with signal locations and relative intensities that correspond to those of the *Standard solution*. In addition, the relative percentage of (1,6) linked glucan is 10%–18% of the total linkages.

COMPOSITION

• **CONTENT OF BETA GLUCAN**

Buffer A: Dissolve 11.6 mL of glacial acetic acid in approximately 900 mL of water. Adjust the solution with 20% sodium hydroxide solution to a pH of 5, and dilute with water to 1000 mL.

Buffer B: Dissolve 69.6 mL of glacial acetic acid in approximately 800 mL of water. Adjust with 20% sodium hydroxide solution to a pH of 3.8, and dilute with water to 1000 mL.

Buffer C: Dissolve 12.12 g of tris(hydroxymethyl) aminomethane (TRIS), 11.69 g sodium chloride, and 4.16 g of ethylenediaminetetraacetic acid (EDTA) tetrasodium salt dihydrate in approximately 900 mL of water. Adjust with concentrated hydrochloric acid or 20% sodium hydroxide solution to a pH of 7.5, and dilute with water to 1000 mL. [NOTE—*Buffer C* can be stored for 1 year at 2°–8°.]

Buffer D¹: Transfer 45.287 g of dibasic potassium phosphate, 30.382 g of *p*-hydroxybenzoic acid, and 4 g of sodium azide into a 1000-mL volumetric flask, and carefully add 800 mL of water. Mix with a stirring bar and mild heat until completely dissolved. Allow the solution to

cool, adjust with 16.7% potassium hydroxide solution to a pH of 7.4, and dilute with water to volume. [NOTE—Store *Buffer D* in an amber bottle with an expiration date of 3 years at 4°.]

Lyticase solution: Prepare the required volume of lyticase from *Arthrobacter luteus*² at a concentration of 10 U/μL by dissolving the quantity stated by the manufacturer (U/mg) in a solution containing 10% (v/v) *Buffer C*. [NOTE—Unused solution can be stored at NMT –15° with an expiration date of 1 year. Every time a different lot of lyticase is used, the concentration of lyticase solution required needs to be qualified.]

(1,6)-Glucanase solution: 1U/300 μL solution of lyophilized (1,6)-glucanase³ in *Buffer A*. [NOTE—Solids may not fully dissolve; therefore, this solution should be handled as a homogeneous suspension. The solution is stable for at least 60 days at NMT –15°.]

Polishing enzyme mix: Mix 2000 U of exo-beta-glucanase⁴ and 400 U of beta-glucosidase⁵ in 100.0 mL of *Buffer A*. A premix of the enzymes may be used as an alternative⁶. [NOTE—Store on ice during the procedure, and for use in a same-day assay. Unused *Polishing enzyme mix* can be refrozen once at NMT –15° with an expiration date of 2 years.]

Glucose oxidase/peroxidase reagent: Dissolve the contents of the Glucose Determination Reagent⁷ in 1 L of water containing 50 mL of *Buffer D*. [NOTE—Store the reagent in an amber bottle, and label with an expiration date of 3 months at a temperature between 2° and 8° or 1 year at NMT –17°. Minimize the time spent at room temperature.]

Sample solution: Transfer 15–20 mg of Beta Glucan into a 16- × 100-mm glass vial. Place the vial in an ice bath. Add a 0.4-mL aliquot of cold potassium hydroxide (1 in 6) while mixing on a vortex mixer to disperse the powder. Return the vial to the ice bath. Continue cycling through mixing on a vortex mixer and placing the vials in the ice bath for 20 min. The mixture should turn into a homogenous, translucent dispersion.

Standard solution: Proceed as directed for the *Sample solution* except replace Beta Glucan with [USP Beta Glucan RS](#). [NOTE—Prepare the *Sample solution* and *Standard solution* in triplicate. It is critical for the success of the assay that the sample is well dispersed.]

Lyticase digestion: Upon removal of all vials containing the *Sample solution* or the *Standard solution* from the ice bath, add 1.6 mL of *Buffer B* and 600 μL of *Lyticase solution* to each vial. Incubate the mixture at 50° for 12–18 h, and cool to room temperature.

(1,6)-Glucanase digestion: After cooling of all vials, remove a 130-μL aliquot of each vial, and digest further by adding 25 μL of 16.7% potassium hydroxide solution and 300 μL of (1,6)-*Glucanase solution*. Incubate vials at 80° for 15 min, and cool to room temperature.

Beta glucanase/glucosidase digestion: After cooling of all vials, add 390 μL of the *Polishing enzyme mix* to each vial, and incubate the vials at 40° for 1 h. Cool them to room temperature, centrifuge, and transfer 50-μL aliquots (in duplicate) to new vials.

Enzyme blank solution: Prepare enzyme blanks in triplicate by combining all the reagents used during the digestion steps except the *Sample solution* or *Standard solution*.

Instrumental conditions

(See [Ultraviolet-Visible Spectroscopy \(857\)](#).)

Mode: Vis

Analytical wavelength: 510 nm

Analysis: Dilute the 50-μL aliquots obtained after the *Beta glucanase/glucosidase digestion* with 50 μL of water, and then add 3 mL of *Glucose oxidase/peroxidase reagent*. Incubate the vials for 20 min at 40°. Determine the absorbance of each vial of the *Sample solution* or *Standard solution* against the *Enzyme blank solution*. Prepare a standard curve using the absorbance of similarly treated series of glucose standards (0, 0.1, 0.25, 0.5, and 1.0 mg/mL). From the slope of the standard curve and the absorbance of the digested *Sample solution* and *Standard solutions*, determine the concentration, *C*, in mg/mL, of liberated glucose in the cuvette:

$$\text{Result} = (A_s - A_b) / \text{slope}$$

A_s = average absorbance of the sample or [USP Beta Glucan RS](#)

A_b = average absorbance of the *Enzyme blank solution*

Calculate the percentage of beta glucan as glucose in the portion of Beta Glucan taken:

$$\text{Result} = 100 \times C / \{[(WT_s / F1) \times (F2 / F3)] / 2\}$$

WT_s = original weight of the sample or [USP Beta Glucan RS](#) (mg)

$F1$ = total volume in the vial during *Lyticase digestion*, 2.6 mL

$F2$ = volume of the sample or [USP Beta Glucan RS](#) transferred to a new vial during (1,6)-*Glucanase digestion*, 0.130 mL

$F3$ = total volume during *Beta glucanase/glucosidase digestion*, 0.845 mL

Acceptance criteria: NLT 70% beta glucan, calculated as glucose after enzymatic hydrolysis, on the dried basis

• CONTENT OF PROTEIN

Sample: 1.0 g of Beta Glucan

Analysis: Proceed as directed in [Nitrogen Determination \(461\)](#), and multiply the nitrogen content by 6.25.

Acceptance criteria: NMT 10.0%

• CONTENT OF FAT

Sample: 2 g of Beta Glucan, previously dried

Analysis: Transfer the *Sample* to an extraction thimble, and mix with an equivalent quantity of dry, clean and Place a fat-free cotton or glass wool plug on top of the thimble. Place the thimble in a continuous-extraction apparatus provided with a tared collection flask. Pour 75 mL