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Validation Report: β-Glucan Assay Kit (Yeast & Mushroom) (cat. no. K-YBGL)

1. Scope

Megazyme's β-Glucan Assay Kit (Yeast & Mushroom) (K-YBGL) is a colourimetric method used for the measurement and analysis of 1,3:1,6-β-D-glucan and α-glucan in yeast and mushrooms. It also measures 1,3-β-D-glucan. This method is a novel method developed in-house and measures Yeast β-Glucan in g/100g on an "as is basis".

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the β-Glucan Assay Kit (Yeast & Mushroom) (K-YBGL).

3. Performance characteristics

The selectivity, working range, limit of detection, trueness (*bias*) and precision of this kit is detailed in this report.

3.1. Selectivity

This assay measures 1,3:1,6-β-D-glucan, 1,3:1,4-β-D-glucan and 1,3-β-D-glucans. Yeast β-glucan does not usually contain 1,3:1,4-β-D-glucan.

This method is not applicable for the analysis of yeast β-glucan in the presence of cellulose (1,4-β-D-glucan) or cereal β-glucans (1,3:1,4-β-glucan). β-glucosidase employed in this kit has varying specificity for β-1,4 and β-1,3 glycosidic bonds, and therefore the cellulose or cereal β-glucans will be partially hydrolysed leading to overestimation of yeast β-glucan.

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3.2. Working Range

The working range for this kit is determined by the D-Glucose control provided in the kit. The glucose measurement (incubation with GOPOD Reagent) is linear between 4 and 100 mg of glucose per assay.

0.1 mL of D-glucose standards at various concentrations is incubated with 3 mL of GOPOD Reagent for 20 min at 40°C. The absorbance values of all samples and blanks read against the reagent blank at 510 nm, as specified in the kit data booklet.

The expected absorbance for 100 µg of the D-glucose control is ~ 1.1. If the absorbance of any sample is higher than that of 100 µg of D-Glucose standard (i.e. higher than 1.1), then the sample must be diluted accordingly.

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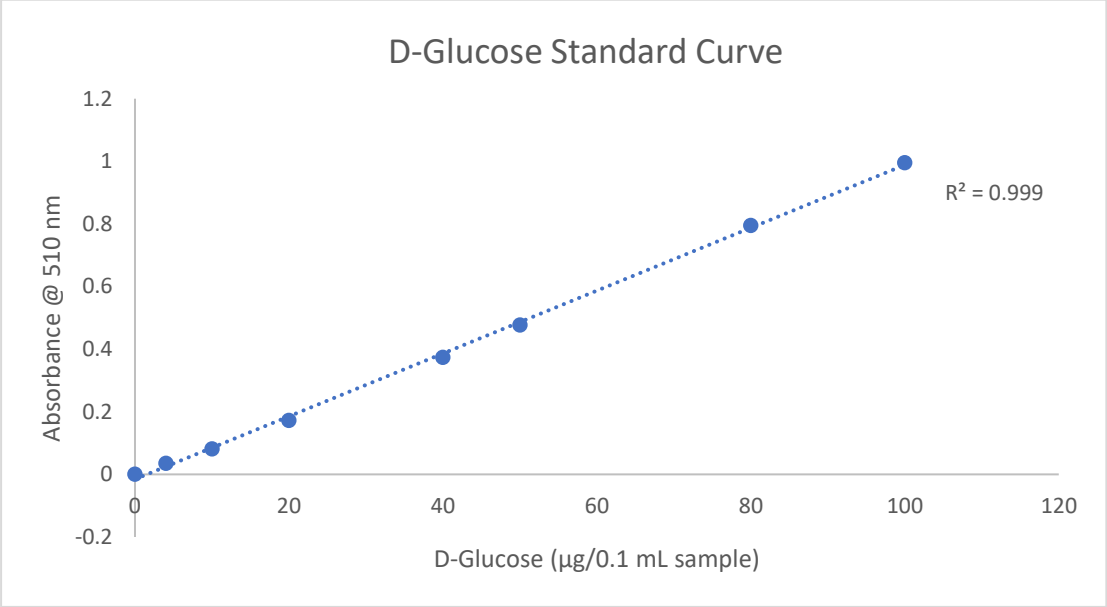
D-Glucose Standard Concentration [µg/0.1 mL]	ΔA_{510nm}
0	0
4	0.03545
10	0.08105
20	0.17165
40	0.3731
50	0.4768
80	0.79435
100	0.9957



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3.3. LOD

If the standard procedure is followed, the smallest differentiating recommended absorbance change (ΔA) is 0.04 (equivalent to ~ 40 µg of D-glucose/mL of sample). This is equivalent to ~ 3. % w/w of β-glucan in the sample, assuming there is no α-glucan present. The highest ΔA should be lower than the absorbance values obtained for 100 µg of the D-glucose standard. This is equivalent to ~ 90% w/w of β-glucan, assuming there is no α-glucan present. If the expected β-glucan is higher the sample should be diluted 2-fold with water, prior to incubation with GOPOD Reagent.

* **Note:** The above detection limits are for samples as used in the assay, after any sample preparations (e.g. deproteinisation). The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the β-Glucan Assay Kit (Yeast & Mushroom) (K-YBGL) method with a suitable reference value (x ref). For this report, Relative Bias is calculated in per cent as: ***b (%)* = x – xref / xref x 100.** The reference material for this purpose is a yeast β-glucan preparation that is supplied with the

β-Glucan Assay Kit (Yeast & Mushroom) (K-YBGL), at ~ 49% β-glucan content.

Relative Bias *b (%)*

	n	Ref Material (% w/w)	Mean (% w/w)	*b*(%)
β-Glucan	23	49	49.7429	1.52

3.5. Precision

This report details the reproducibility of the β-Glucan Assay Kit (Yeast & Mushroom) (K-YBGL), it is a measure of the variability in results, on different days and by different analysts, over an extended period of time.
For the purpose of this report different lot numbers of the kit standard is used as the reference material.

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Reproducibility

	n	Ref Material (%w/w)	Mean (%w/w)	Standard Deviation	%CV
β-Glucan	23	49	49.7429	0.6530	1.31

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of Yeast β-glucan in yeast and mushroom matrices and has been used for many years. Data presented in this report verifies and validates that this method is fit for the purpose intended and is summarised below.

Validation Summary	Glucose
Working range (µg in assay)	4-100
LOD (ΔA)	0.04
Relative Bias *b* (%)	1.52
Reproducibility (%CV using yeast β-glucan)	1.31