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Validation Report: Enzymatic Yeast β-Glucan Assay Kit (cat. no. K-EBHLG)

1. Scope

Megazyme's Enzymatic Yeast β-Glucan Assay Kit (K-EBHLG), is an enzymatic method used for the measurement and analysis of 1,3:1,6-β-glucan in yeast it also measures 1,3-β-glucan. This method is a novel method developed in-house and measures Yeast β-Glucan in g/100g on an "as is basis" - if moisture content is known, β-Glucan can be measured in g/100g on a "dry weight basis" also.

2. Planning

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

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. The method gives quantitative measurement of β-glucan in curdlan, laminarin and cereal β-glucan preparations. Yeast β-glucan does not usually contain 1,3:1,4-β-D-Glucan.

This method does not give quantitative measurement of β-glucan in mushrooms, the reasons for which are currently being researched. It is also not suitable for analysis of wine, baby formula or scleroglucan as it is not sensitive enough.

This method is also not applicable for the analysis of yeast β-glucan in the presence of cellulose (1,4-β-D-glucan). β-glucosidase employed in this kit has varying specificity for β-1,4 and β-1,3 glycosidic bonds, and therefore the cellulose 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 150 µg of glucose per assay.

0.1 mL of D-glucose standards at various concentrations incubated with 4 mL of GOPOD Reagent for 20 min at 40°C. The absorbances read against the reagent blank at 510 nm, as specified in the kit data booklet.

The absorbance for 150 µg is ~ 1.1. If the absorbance of your samples is higher than that of 150 µg of D-Glucose control (i.e. higher than 1.1) they must be diluted accordingly.

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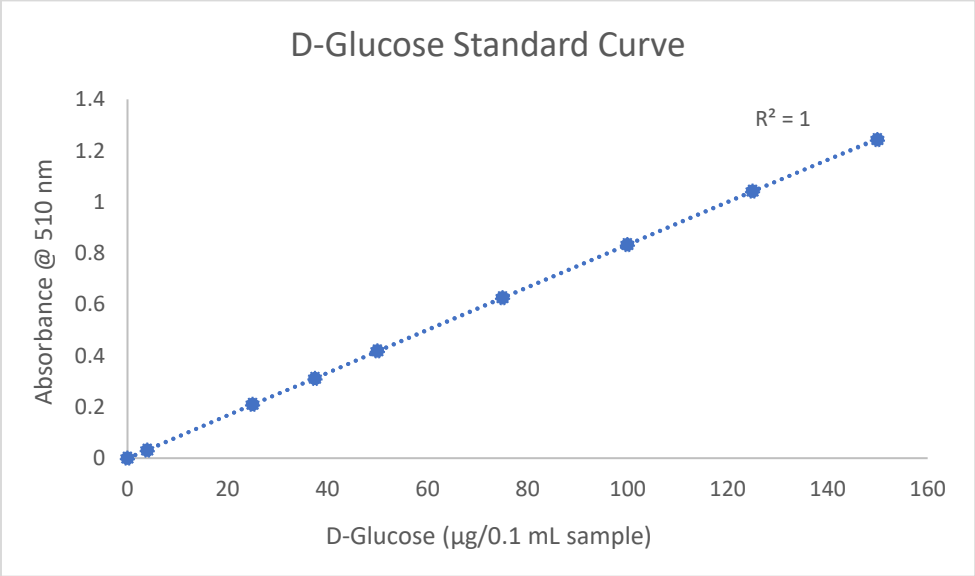
D-Glucose Standard Concentration [µg/0.1 mL]	ΔA_{510nm}
0	0
4	0.0308
25	0.2094
37.5	0.31075
50	0.41815
75	0.6264
100	0.83335
125	1.0421
150	1.24325



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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 ~ 2.7% of β-glucan in the sample. The highest ΔA should be lower than the absorbance values obtained for 150 µg of glucose. This is equivalent to ~ 80% of β-glucan. 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 sample preparations if required (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 Enzymatic Yeast β-Glucan Assay Kit (K-EBHLG) 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 fungal β-glucan preparation supplied with the Enzymatic Yeast β-Glucan Assay Kit (K-EBHLG), at 46% β-glucan content.

Relative Bias *b(%)*

	n	Ref Material (% w/w)	Mean (% w/w)	*b*(%)
β-Glucan	16	46	45.86	-0.31

3.5. Precision

This report details the reproducibility of the Enzymatic Yeast β-Glucan Assay Kit (K-EBHLG), 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.

Reproducibility

	n	Ref Material (%w/w)	Mean (%w/w)	Standard Deviation	%CV
β-Glucan	16	46	45.86	0.5005	1.09

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of Yeast β-glucan in various matrices. It has been used for many years and is fully automatable for high throughput analysis of samples. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	Glucose
Working range (µg in assay)	4-150
LOD (ΔA)	0.04
Relative Bias *b*(%)	- 0.31
Reproducibility (%CV using fungal β-glucan)	1.09