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Validation Report: β-Glucan Assay Kit (Mixed Linkage) (cat. no. K-BGLU)

1. Scope

Megazyme's β-Glucan Assay Kit (Mixed Linkage) (K-BGLU) is a colorimetric method used for the measurement and analysis of 1,3:1,4-β-D-glucan in cereal grains, milling fractions, wort, beer and other food products. The Streamlined β-glucan method has been successfully evaluated by AOAC International (Method 995.16), AACC (Method 32-23.01) and ICC (Method No. 166). The original version of the method was also successfully evaluated by Analytical Committees of the Royal Australian Chemical Institute, CODEX (Type II Method) and the European Brewing Convention (Methods 3.10.1, 4.16.1 and 8.13.1). This method measures 1,3:1,4-β-D-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 (Mixed Linkage) (K-BGLU).

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 is specific for mixed linkage 1,3:1,4-β-D-glucan.

3.2. Working Range

The working range for the β-Glucan Assay Kit (Mixed Linkage) (K-BGLU) is determined by the D-Glucose control provided in the kit.

The glucose measurement (incubation with GOPOD Reagent) is linear between 4 to 100 µg of D-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 are read against the reagent blank at 510 nm, as specified by the kit data booklet.

The 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 control (i.e. higher than 1.1) the sample must be diluted accordingly.

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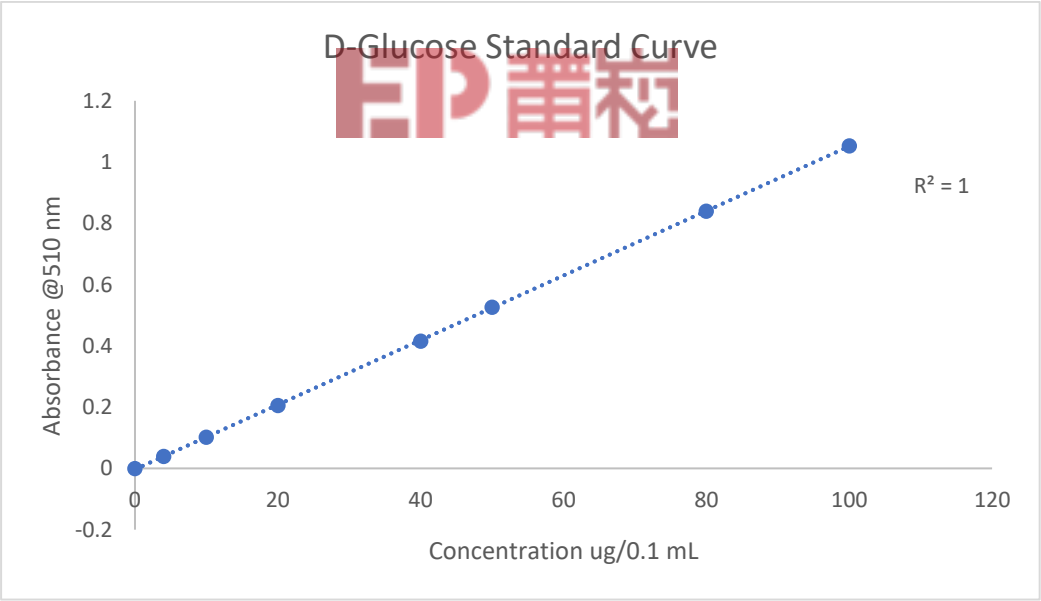
D-Glucose Standard Concentration [µg/0.1 mL]	ΔA_{510nm}
0	0
4	0.0393
10	0.10175
20	0.2054
40	0.4152
50	0.52675
80	0.8395
100	1.0529



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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 ~ 0.35% w/w of β-glucan in the sample. The highest ΔA should be lower than the absorbance values obtained for 100 µg of the D-glucose standard. This is equivalent to ~ 8.5% w/w of β-glucan. If the expected β-glucan content is higher the sample solution should be diluted sufficiently with 200 mM sodium acetate buffer (pH 4.0) before the β-glucosidase treatment.

* **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 β-Glucan Assay Kit (Mixed Linkage) (K-BGLU) 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 standardised oat flour control, supplied with the β-Glucan Assay Kit (Mixed Linkage) (K-BGLU) at 7.5% w/w.

Relative Bias *b(%)*

	n	Ref Material (% w/w)	Mean (% w/w)	*b*(%)
β-glucan	12	7.5	7.518	0.24

3.5. Precision

This report details the reproducibility of the β-Glucan Assay Kit (Mixed Linkage) (K-BGLU), 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 oat standard is used as the reference material.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
β-glucan	12	7.5	7.518	0.0542	0.72

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of mixed linkage β-glucan in various matrices. It has been used for many years and is widely used and accepted in clinical chemistry and food analysis. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	D-Glucose
Working range (µg/0.1 mL)	4-100
LOD (ΔA)	0.04
Relative Bias *b*(%),(using oat standard β-glucan)	6.10
Reproducibility (%CV using oat standard β-glucan)	0.52