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Validation Report: D-Glucose Assay Kit (GOPOD Format) (cat. no. K-GLUC)

1. Scope

Megazyme's D-Glucose Assay Kit (GOPOD Format) (K-GLUC) is a colourimetric method which employs high purity glucose oxidase and peroxidase. This kit can be used with confidence for the specific measurement of D-glucose in foodstuffs, beverages and other materials. This method measures D-glucose in µg/0.1 mL from a reference standard and is widely used and accepted in clinical chemistry and food analysis.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the D-Glucose Assay Kit (GOPOD Format) (K-GLUC).

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 D-glucose.

Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding D-glucose to the sample in the initial extraction steps.

3.2. Working Range

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

Assay follows the D-Glucose Assay Kit (GOPOD Format) (K-GLUC) standard procedure. 0.1 mL of D-glucose standard at various concentrations (0.04-1.0 g/L D-glucose) incubated with 3 mL of GOPOD Reagent, which corresponds to 4-100 µg of D-glucose per reaction. Absorbance A2 was read at 510 nm after incubation at 40-50°C for 20 min as recommended in the procedure.

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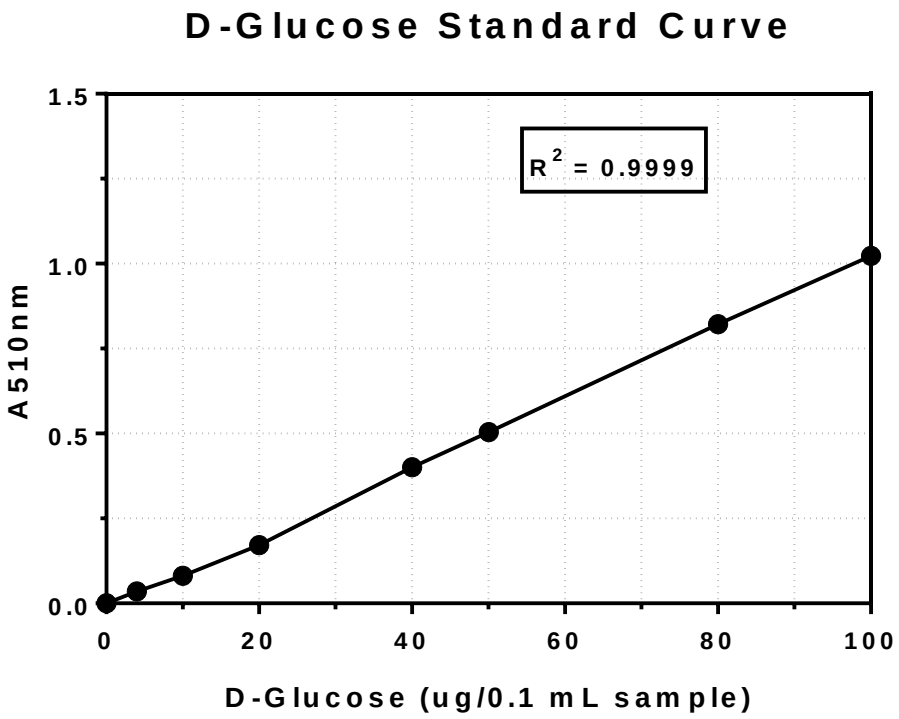

3.3. LOD

If the standard procedure is followed, the smallest differentiating recommended absorbance change (ΔA) is 0.04 (equivalent to ~ 4 µg of D-glucose / 0.1 mL of sample). The highest ΔA should be lower than the absorbance values obtained for 100 µg of D-glucose.

* **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 D-Glucose Assay Kit (GOPOD Format) (K-GLUC) 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 D-glucose, supplied with the D-Glucose Assay Kit (GOPOD Format) (K-GLUC) at 1.0 g/L.

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Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
D-Glucose	15	1.0	1.0610	6.10

3.5. Precision

This report details the reproducibility of the D-Glucose Assay Kit (GOPOD Format) (K-GLUC), 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 (g/L)	Mean (g/L)	Standard Deviation	%CV
D-Glucose	12	1.0	1.0610	0.0055	0.52

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-glucose 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 (mg/mL)	0.04
Relative Bias *b*(%)	6.10
Reproducibility (%CV using D-glucose)	0.52