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Validation Report: Sucrose/D-Glucose Assay Kit (cat. no. K-SUCGL)

1. Scope

Megazyme's Sucrose/D-Glucose Assay Kit (K-SUCGL) employs high purity glucose oxidase, peroxidase and β -fructosidase (invertase) and can be used with confidence for the specific measurement of D-glucose and sucrose in various materials including foods and food extracts, plants, beverages, honey and other materials. This method measures glucose in $\mu\text{g}/0.1\text{ mL}$ 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 Sucrose / D-Glucose Assay Kit (K-SUCGL).

3. Performance characteristics

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

3.1. Selectivity

The β -fructosidase employed in this assay is specific for sucrose. The high purity glucose oxidase employed is specific for glucose.

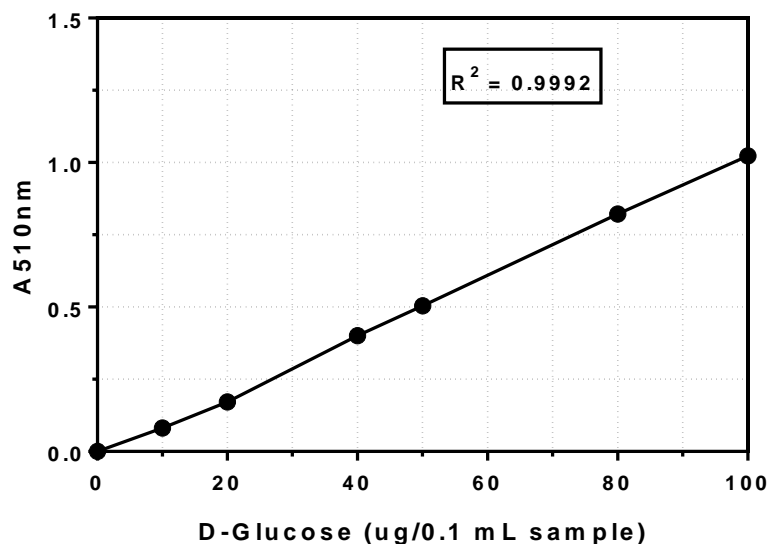
Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding sucrose/glucose to the sample in 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 glucose measurement (incubation with GOPOD Reagent) is linear between 10 and 100 μg of D-glucose per assay.

Assay follows the Sucrose/D-Glucose Assay Kit (K-SUCGL) standard procedure. 0.1 mL of glucose standard at various concentrations (0.1-1.0 g/L glucose) were incubated with 0.3 mL of distilled water + 3 mL of GOPOD Reagent, which corresponds to 10-100 μg of glucose per reaction. Absorbance was read at 510 nm after incubation at 40-50°C for 20 min as recommended in the procedure.

D-Glucose Standard Curve



3.3. LOD and LOQ

If the standard procedure is followed, the smallest differentiating recommended absorbance change (ΔA) is 0.04 (equivalent to $\sim 2 \mu\text{g}$ of D-glucose/0.2 mL of sample). The highest ΔA should be lower than the absorbance values obtained for 100 μg of glucose.

* **Note:** The above detection limits are for samples as used in the assay, after any sample preparation, if required. 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 Sucrose / D-Glucose Assay Kit (K-SUCGL) with a suitable reference value (x_{ref}). For this report, Relative Bias is calculated in per cent as: $b(\%) = \frac{x - x_{\text{ref}}}{x_{\text{ref}}} \times 100$. The reference material for this purpose is the control flour sample supplied with the Sucrose/D-Glucose Assay Kit (K-SUCGL) with a sucrose content of 26.8 % (w/w) and D-glucose content of 10.2 % (w/w).



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Relative Bias *b*(%)

	n	Ref Material (% w/w)	Mean (% w/w)	<i>b</i> (%)
Sucrose	20	26.8	26.4	-1.39
D-Glucose	20	10.2	9.76	-4.33

3.5. Precision

This report details the reproducibility of the Sucrose/D-Glucose Assay Kit (K-SUCGL), it is a measure of the variability in results on different occasions, by different analysts, over an extended period of time.

Reproducibility

	n	Ref Material (% w/w)	Mean (% w/w)	Standard Deviation	% CV
Sucrose	20	26.8	26.4	0.2758	1.04
D-Glucose	20	10.2	9.76	0.2152	2.20

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of Sucrose/D-Glucose in various matrices. Data presented in this report verifies and validates that this method is fit for the purpose intended, which is summarised below.

Validation Summary	Sucrose	D-Glucose
Working range ($\mu\text{g} / 0.2 \text{ mL}$)	10-100	10-100
LOD (mg/mL)	0.04	0.04
Relative Bias <i>b</i> (%)	-1.39	-4.33
Reproducibility (%CV using kit control flour)	1.04	2.20