Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

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

1. Scope

Megazyme's Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG) is an enzymatic method used for the measurement and analysis of sucrose, D-fructose and D-glucose in plant and food products. This method was developed in-house and measures each sugar in g/L. Methods based on this principle have been accepted by NF, EN, NEN, DIN, GOST, IFU, AIJN, MEBAK and IOCCC.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG).

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 assays are specific for D-glucose and D-fructose. Since β-fructosidase also hydrolyses low molecular weight fructans (e.g. kestose) this method, as with all others, is not absolutely specific for sucrose.

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, D-fructose and, or, D-mannose to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG) standard procedure. 0.1 mL of D-glucose plus D-fructose mixed standard was used as sample, with a range of concentrations (0.04-0.8 g/L total sugars) which corresponds to 4-80 µg of total sugars per cuvette. In the case of the supplied kit standard, with no sucrose present, it is sufficient to carry out D-glucose and D-fructose analysis only.

Absorbance A2 was taken 5 min after the addition of the 1st trigger enzyme (HK/G6P-DH), giving the measurement of D-glucose.

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

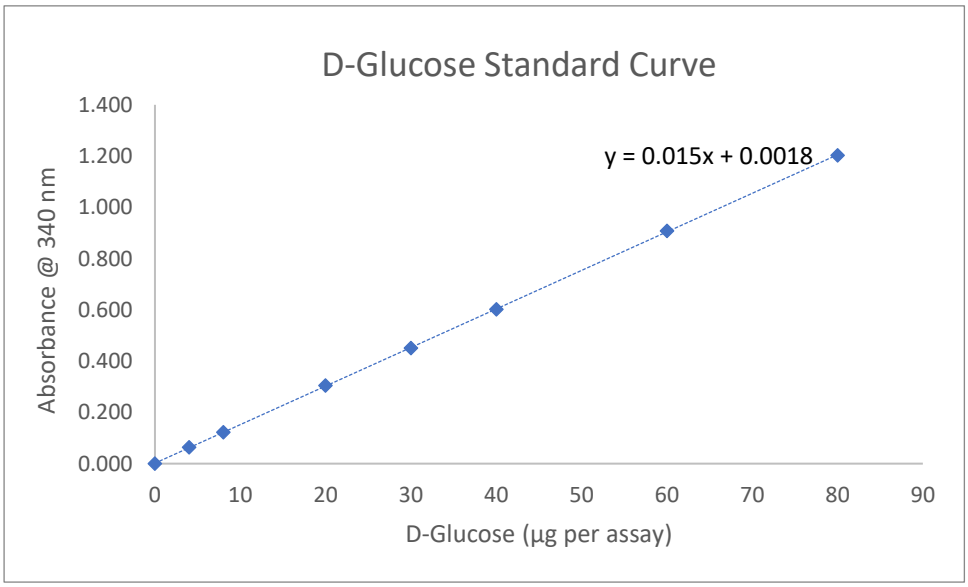
Absorbance A3 was taken 10 min after the addition of the trigger enzyme (PGI), giving the measurement of D-fructose.

For sucrose, a commercially available sucrose solution can be used to confirm the effectiveness of β-fructosidase enzyme. The sucrose is hydrolysed to D-glucose and D-fructose. The total D-glucose following hydrolysis by β-fructosidase is then measured by HK/G6P-DH enzyme, after 5 min. The sucrose content is then calculated from the difference in D-glucose concentrations before and after hydrolysis by β-fructosidase.
As sucrose is hydrolysed to D-glucose and D-fructose the linearity of sucrose is not detailed in this report.

All absorbances were read at 340 nm and at 25°C as recommended in the procedure.

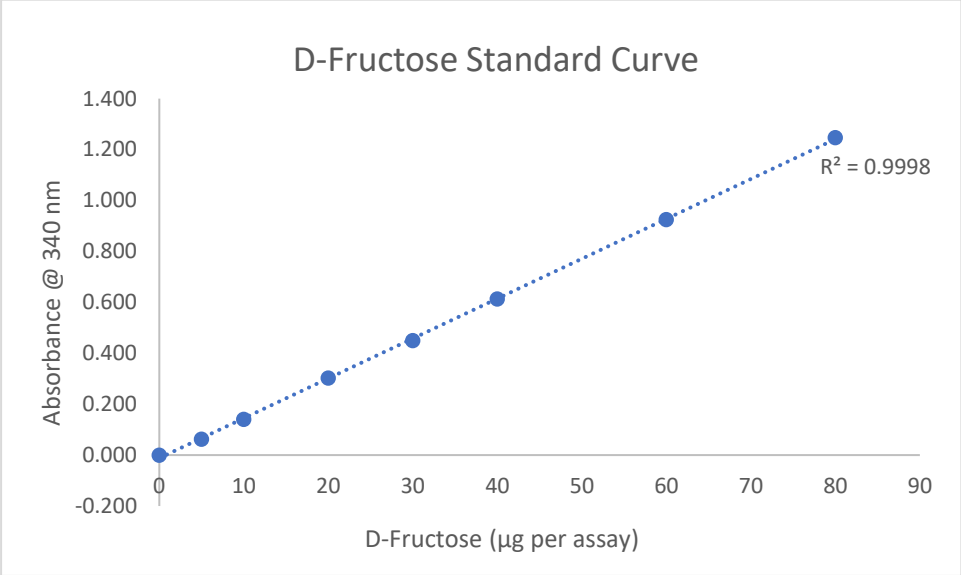
Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

D-Glucose Concentration [µg/assay]	ΔA_{340nm}
0	0.000
4	0.063
8	0.122
20	0.305
30	0.451
40	0.602
60	0.908
80	1.203



Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

D-Fructose Concentration [µg/assay]	ΔA_{340nm}
0	0.000
5	0.063
10	0.141
20	0.303
30	0.450
40	0.613
60	0.925
80	1.247



Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 1.38 mg/L, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 1.00 mL.

The **calculated limit of detection (LOD)** and the calculated **limit of quantification (LOQ)** for this report purpose is based on the analysis of samples that have been taken through the whole Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG) procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as 3 x s'0; where s'0 is the standard deviation of a number of samples A1 reading.
- The LOQ is the lowest level at which the kit's performance is acceptably repeatable. LOQ is calculated as kQ x s'0; where s'0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for kQ is 10.

- For Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG)

  LOD – For 1.0 mL of sample (maximum volume)
  D-Glucose = 1.4 mg/L
  D-Fructose = 1.4 mg/L
  Sucrose = 0.200 mg/L

  LOQ – For 1.0 mL of sample (maximum volume)
  D-Glucose = 4.2 mg/L
  D-Fructose = 5.6 mg/L
  Sucrose = 0.500 mg/L

* **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.

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG) 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 is for this purpose is D-glucose and D-fructose, supplied with the Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG) at 0.2 g/L of each sugar and also commercially available sucrose at a concentration of 0.8 g/L.

Relative Bias *b(%)*

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
D-Glucose	10	0.2	0.2018	0.89
D-Fructose	10	0.2	0.1994	-0.28
Sucrose	9	0.8	0.7966	-0.43

3.5. Precision

This report details the reproducibility of the Sucrose/D-Fructose/D-Glucose Assay Kit (K-SUFRG), 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 are used as the reference material, for D-glucose plus D-fructose, while commercially available sucrose made to 0.8 g/L is used as the reference material for sucrose.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
D-Glucose	10	0.2	0.2018	0.0015	0.76
D-Fructose	10	0.2	0.1994	0.0017	0.87
Sucrose	9	0.8	0.7966	0.0019	0.24

Megazyme,
Bray Business Park,
Bray, Co. Wicklow,
A98 YV29,
Ireland.
Tel: + 353 1 286 1220
Fax: + 353 1 286 1264

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-glucose, D-fructose and sucrose 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	D-Glucose	D-Fructose	Sucrose
Working range (µg in cuvette)	4-80	4-80	4-80
LOD (mg/L)	1.4	1.4	0.2
LOQ (mg/L)	4.2	5.6	0.5
Relative Bias *b*(%)	0.894	-0.284	-0.425
Reproducibility (%CV)	0.76	0.87	0.24