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

Validation Report: Trehalose Assay Kit (cat. no. K-TREH)

1. Scope

Megazyme's Trehalose Assay Kit, (K-TREH) is an enzymatic method used for the rapid measurement and analysis of trehalose in foodstuffs, beverages and other materials. This novel trehalose method was developed in-house and measures trehalose in g/L.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by Trehalose Assay Kit (K-TREH).

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

This assay is specific for trehalose.

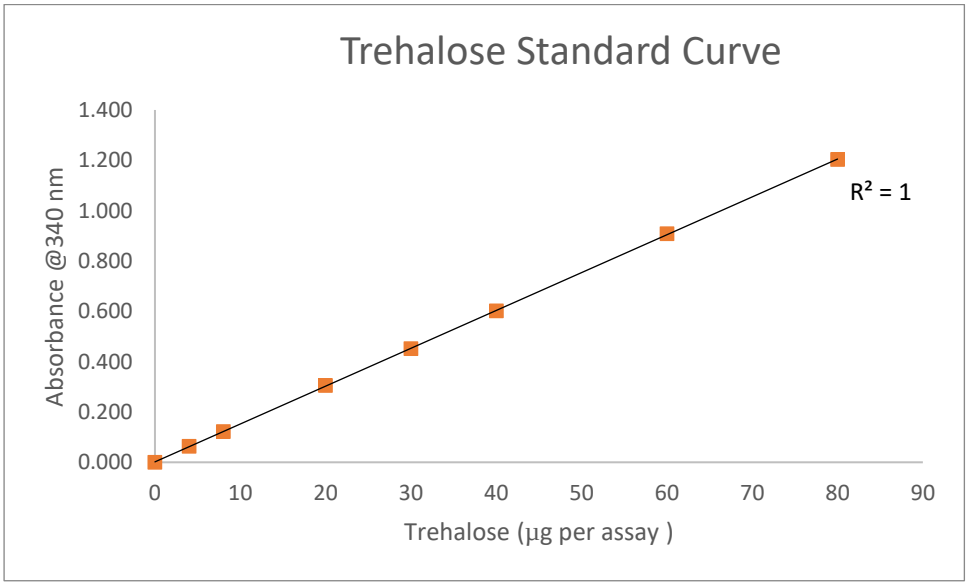
Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding trehalose to the sample in the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

3.2. Working Range

Assay follows the Trehalose Assay Kit (K-TREH) standard procedure. 0.2mL of Trehalose standard was used as sample, with a range of concentrations (0.02-0.4 g/L Trehalose) which corresponds to 4-80 µg of trehalose per assay. Absorbance A2 was read after 5 min at 340nm and at 25°C as recommended in the standard assay procedure.

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

Trehalose 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

3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, (reduced assay procedure) is 37.52 mg/L which is derived from an absorbance difference of 0.020 with a sample volume of 0.2 mL.
The **instrument limit of detection** of samples (non-reduced assay procedure) is 6.9 mg/L which is derived from an absorbance difference of 0.020 with a sample volume of 0.2 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 Trehalose Assay Kit (K-TREH) measurement 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 k_Q x s'_0; where s'_0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for k_Q is 10.

- For Trehalose Assay Kit (K-TREH)

  LOD – For 2.0mL of sample (maximum volume) (non-reduced procedure)
  Trehalose = 0.069 mg/L

  LOQ – For 2.0mL of sample (maximum volume) (non-reduced procedure)
  Trehalose = 0.276 mg/L

* **Note:** The above detection limits are for samples as used in the assay, after sample preparation, 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 Trehalose Assay Kit (K-TREH) 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 Trehalose supplied with the Trehalose Assay Kit (K-TREH) at 0.18 g/L.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
Trehalose	18	0.18	0.1807	0.4

3.5. Precision

This report details the reproducibility of the Trehalose Assay Kit (K-TREH), 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
Trehalose	18	0.18	0.1807	0.002	1.09

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 Trehalose 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	Trehalose
Working range (µg in cuvette)	4-80
LOD (mg/L)	0.069*
LOQ (mg/L)	0.276*
Relative Bias *b* (%)	0.4
Reproducibility (%CV using kit standard)	1.09

*non-reduced procedure used