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

Validation Report: *myo*-Inositol Assay Kit (cat. no. K-INOSL)

1. Scope

Megazyme's *myo*-Inositol Assay Kit (K-INOSL) is a reliable and accurate enzymatic UV-method for the specific measurement and analysis of *myo*-inositol in animal feeds, foods and various other materials. This novel *myo*-inositol method was developed in-house and measures *myo*-inositol in g/L.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by *myo*-Inositol Assay Kit (K-INOSL).

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 assay rapidly converts myo-inositol and also converts D-glucose and D-xylose. However, D-glucose is phosphorylated during the procedure by hexokinase to D-glucose 6-phosphate which does not react.

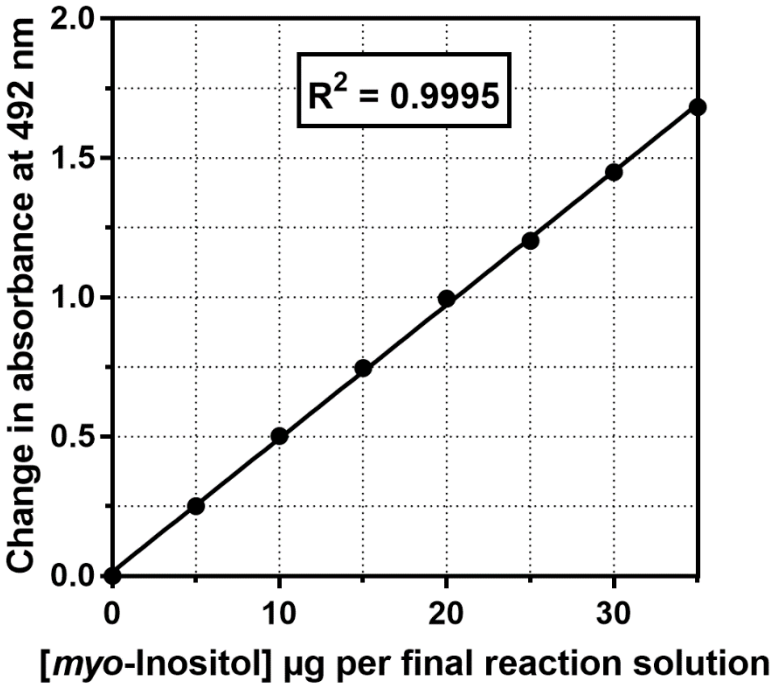
Interfering substances in the sample being analysed can be identified by including an internal standard. Quantitative recovery of this standard would be expected. Losses in sample handling and extraction are identified by performing recovery experiments, i.e. by adding *myo*-inositol to the sample in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

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

3.2. Working Range

Assay follows the *myo*-Inositol Assay Kit (K-INOSL) standard procedure. 0.1 mL of *myo*-inositol standard was used as a sample, with a range of concentrations (0.02-0.35 g/L) which corresponds to 2-35 µg of *myo*-inositol per assay. Absorbance A2 was read after 15 min, at 492 nm and at 25°C as recommended in the procedure.

The working range is linear between 2-35 µg of *myo*-inositol per assay.



3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.82 mg/L of *myo*-inositol, which is derived from an absorbance difference of 0.020 with a maximum sample volume of 0.5 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 standard procedure of the m*yo*-Inositol Assay (K-INOSL).

- The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as 3 x σ of the blank sample solution absorbance and 10 x σ of the blank sample

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

solution absorbance, respectively, where σ is the standard deviation of the absorbance values from (at least) 10 replicates.

- For m*yo*-Inositol Assay Kit (K-INOSL)

  LOD – For 0.5 mL of sample (maximum volume)
  myo-Inositol = 0.164 mg/L

  LOQ – For 0.5 mL of sample (maximum volume)
  myo-Inositol = 0.573 mg/L

* **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 *myo*-Inositol Assay Kit (K-INOSL) 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 *myo*-inositol supplied with the m*yo*-Inositol Assay Kit (K-INOSL) at 0.25 g/L.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
***myo*-Inositol**	22	0.25	0.2485	-0.62

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

3.5. Precision

This report details the reproducibility of the *myo*-Inositol Assay Kit (K-INOSL), 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 (g/L)	Mean (g/L)	Standard Deviation	%CV
***myo*-Inositol**	22	0.25	0.2485	0.0026	1.06

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of *myo*-inositol 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	*myo*-Inositol
Working range (µg in cuvette)	2 - 35
LOD (mg/L)	0.164
LOQ (mg/L)	0.573
Relative Bias *b*(%)	-0.62
Reproducibility (%CV using kit standard)	1.06