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Validation Report: L-Glutamic Acid Assay Kit (cat. no. K-GLUT)

1. Scope

Megazyme's L-Glutamic Acid Assay Kit (K-GLUT) is an enzymatic method used for the rapid measurement and analysis of L-glutamic acid (MSG) in foodstuffs. This method was developed in-house and measures L-glutamic acid in g/L. Methods based on this principle have been accepted by ISO, GOST and NMKL.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by L-Glutamic Acid Assay Kit (K-GLUT).

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 L-glutamic acid.

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 ammonia to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the L-Glutamic Acid Assay Kit (K-GLUT) standard procedure. 0.1 mL of L-glutamic acid standard was used as sample, with a range of concentrations (0.004-0.2 g/L ammonia) which corresponds to 0.4-20 µg of L-glutamic acid per cuvette. Absorbance A2 was read after ~ 10 min, at 492 nm and at 25°C as recommended in the procedure.

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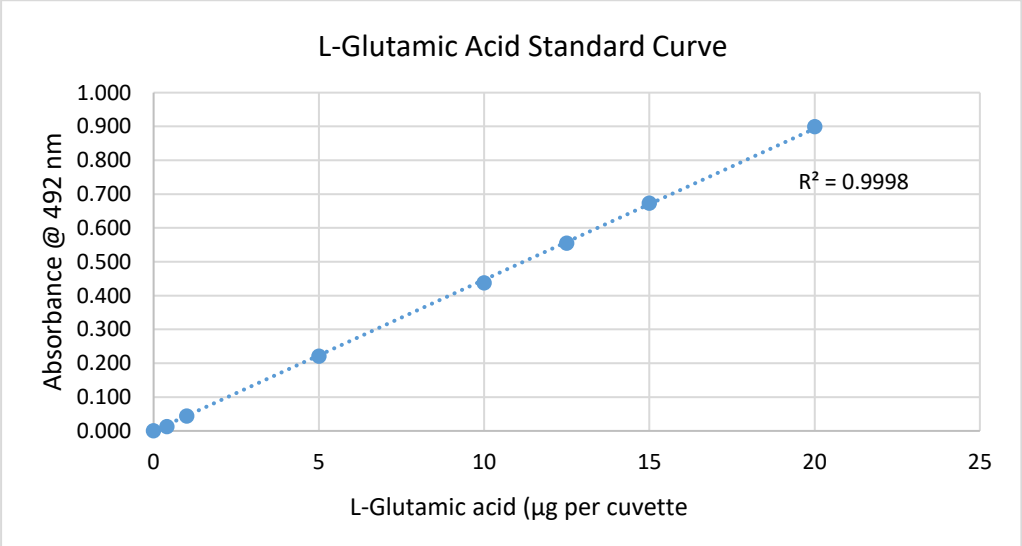
L-Glutamic Acid Concentration [µg/cuvette]	ΔA_{492nm}
0	0.000
0.4	0.013
1	0.044
5	0.221
10	0.438
12.5	0.555
15	0.673
20	0.899



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.214 mg/L, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 2.00 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 L-Glutamic Acid Assay Kit (K-GLUT) 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 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 L-Glutamic Acid Assay Kit (K-GLUT)

  LOD – For 2.0 mL of sample (maximum volume)
  L-Glutamic acid = 0.075 mg/L

  LOQ – For 2.0 mL of sample (maximum volume)
  L-Glutamic acid = 0.247 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.

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3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with L-Glutamic Acid Assay Kit (K-GLUT) 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 L-glutamic acid supplied with the L-Glutamic Acid Assay Kit (K-GLUT) at 0.1 g/L.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
L-Glutamic Acid	18	0.1	0.0997	-0.25

3.5. Precision

This report details the reproducibility of the L-Glutamic Acid Assay Kit (K-GLUT), 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
L-Glutamic Acid	18	0.1	0.0997	0.0022	2.22

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of L-glutamic acid 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	L-Glutamic Acid
Working range (µg in cuvette)	0.4-20
LOD (mg/L)	0.75
LOQ (mg/L)	0.247
Relative Bias *b* (%)	-0.25
Reproducibility (%CV using kit standard)	2.22