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Validation Report: L-Asparagine/L-Glutamine/Ammonia Assay Kit (Rapid) (cat. no. K-ASNAM)

1. Scope

Megazyme's L-Asparagine/L-Glutamine/Ammonia Assay Kit (K-ASNAM), is an enzymatic method used for the convenient, cost effective and rapid measurement and analysis of L-asparagine/L-glutamine/ammonia as acrylamide precursors in the food industry, or as cell culture media/supernatant components, or in other materials. This method is a novel method, developed in-house and measures each analyte in g/L.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by L-Asparagine/L-Glutamine/Ammonia Assay Kit (K-ASNAM).

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 is specific for L-asparagine, L-glutamine and free ammonium ions. The D-isomers do 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 ammonia, L-asparagine and, or, L-glutamine to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the L-Asparagine/L-Glutamine/Ammonia Assay Kit (K-ASNAM) standard procedure. 0.1 mL of ammonia standard solution was used as sample, with a range of concentrations (0.002-0.07 g/L) which corresponds to 0.2-7.0 µg of ammonia per cuvette.

The L-asparagine standard was also prepared and 0.1 mL was tested at a range of concentrations (0.005-0.5 g/L) which corresponds to 0.5-50 µg of L-asparagine per cuvette.

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Similarly, a L-glutamine standard was prepared and 0.1 mL was tested at a range of concentrations (0.005-0.5 g/L) which corresponds to 0.5-50 µg of L-glutamine per cuvette.

Glutaminase enzyme was used initially for 5 min in the L-glutamine/L-asparagine (GLN/ASN) reaction to convert L-glutamine into L-glutamate and ammonium ions. A1 of all reactions (ammonia and GLN/ASN) is then recorded.

Absorbance A2 was taken 5 min after the addition of the trigger enzyme GLDH for all reactions, giving the measurement of ammonia in the ammonia reaction, and the measurement of L-glutamine + ammonia in the GLN/ASN reaction. Subsequently, L-glutamine content can be calculated by subtracting the absorbance received from Ammonia reading from the absorbance received from the L-glutamine + ammonia reading and applying to the given calculation.

Absorbance A3 was taken 5 min after the addition of the trigger enzyme (asparaginase) for the GLN/ASN reaction only, giving the measurement of L-asparagine.

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

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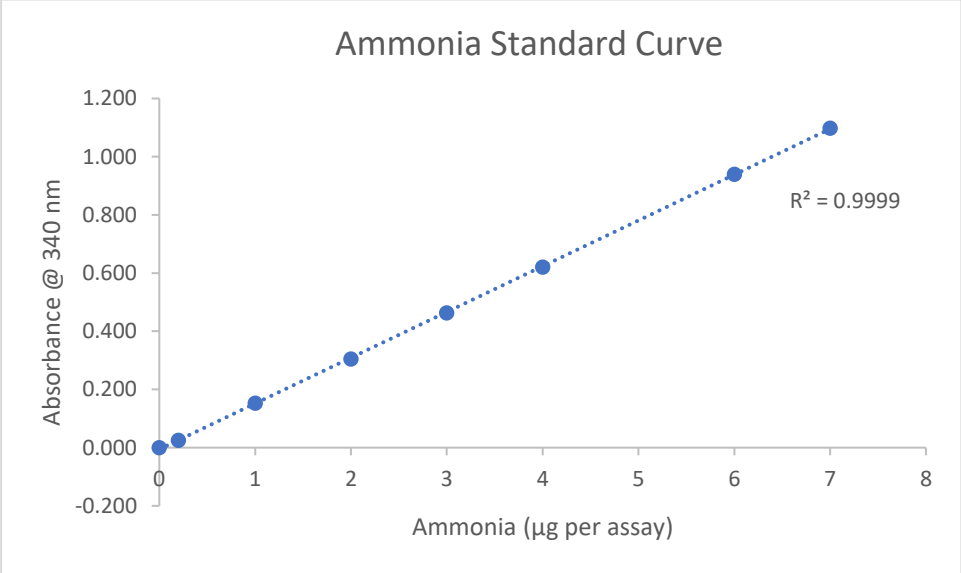
Ammonia Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.2	0.025
1	0.153
2	0.305
3	0.463
4	0.620
6	0.940
7	1.098



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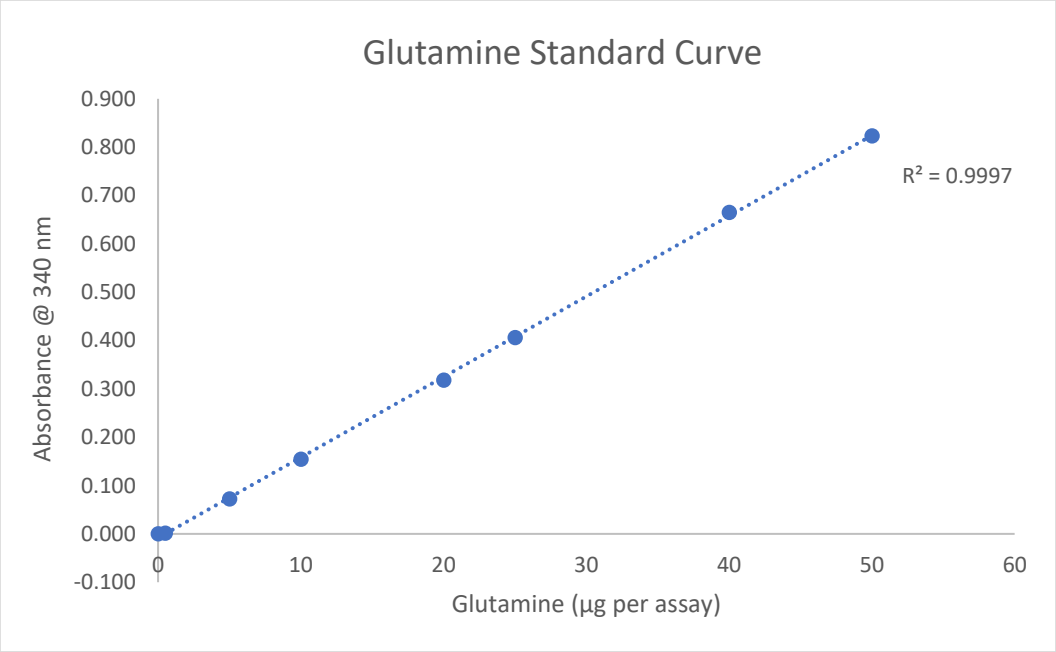
Glutamine Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.5	0.001
5	0.072
10	0.154
20	0.318
25	0.406
40	0.665
50	0.823



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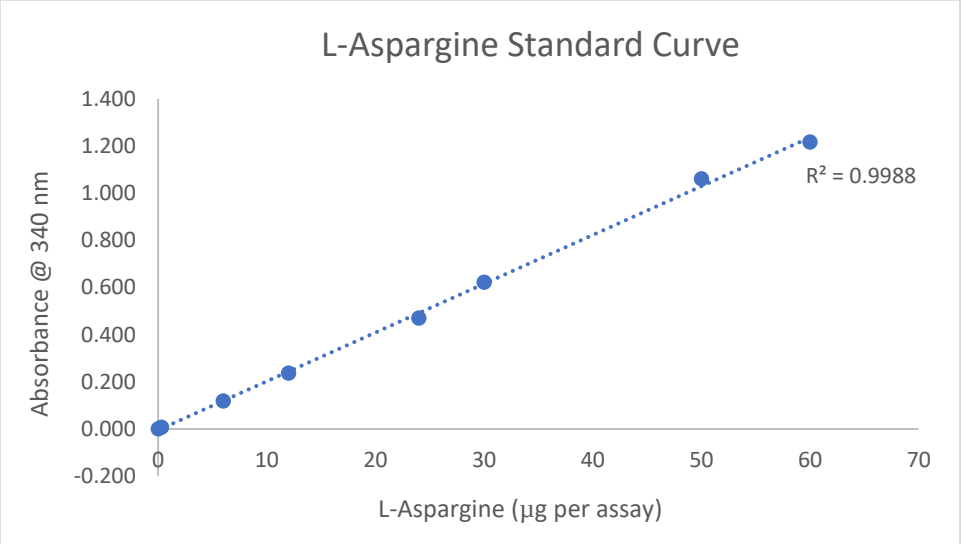
L-Asparagine Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.3	0.007
6	0.119
12	0.237
24	0.470
30	0.622
40	1.060
50	1.217



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet is 0.127 mg/L ammonia, 1.09 mg/L L-glutamine or 0.99 mg/L L-asparagine, which are 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 L-Asparagine/L-Glutamine/Ammonia Assay Kit (K-ASNAM) 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-Asparagine/L-Glutamine/Ammonia Assay Kit (K-ASNAM)

  LOD – For 1.5 mL of sample (maximum volume)

  Ammonia = 0.9 mg/L
  L-Asparagine = 1.5 mg/L
  L-Glutamine = 1.6 mg/L

  LOQ – For 1.5 mL of sample (maximum volume)
  Ammonia = 2.8 mg/L
  L-Asparagine = 4.9 mg/L
  L-Glutamine = 3.6 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-Asparagine/L-Glutamine/Ammonia Assay Kit (K-ASNAM) 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**, where the reference material is ammonia at 0.04 g/L and L-asparagine at 0.3g/L both which are supplied with the L-Asparagine/L-Glutamine/Ammonia Assay Kit (K-ASNAM).

Relative Bias *b(%)*

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
Ammonia	13	0.04	0.0402	-0.5
L-Asparagine	12	0.3	0.2941	-1.97

3.5. Precision

This report details the reproducibility of the L-Asparagine/L-Glutamine/Ammonia Assay Kit (K-ASNAM), 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 ammonia and L-asparagine kit standards are used as the reference materials.

Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
Ammonia	13	0.04	0.0402	0.0006	1.5
L-Asparagine	12	0.3	0.2941	0.0027	0.93

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of L-asparagine, L-glutamine and ammonia 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	Ammonia	L-Asparagine	L-Glutamine
Working range (µg in cuvette)	0.2-7.0	0.5-50	0.5-50
LOD (mg/L)	0.9	1.5	1.6
LOQ (mg/L)	2.8	4.9	3.6
Relative Bias *b*(%)	0.5	-1.97	-
Reproducibility (%CV)	1.5	0.93	-