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

Validation Report: Urea/Ammonia Assay Kit (Rapid) (cat. no. K-URAMR)

1. Scope

Megazyme's Urea/Ammonia Assay Kit (K-URAMR) is an enzymatic method used for the measurement and analysis of Urea and Ammonia in water, beverages, milk and food products. This method was developed in-house and measures both Urea and Ammonia in g/L. Methods based on this principle have been accepted by NEN and MEBAK.

2. Planning

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

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 urea and ammonia.

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

3.2. Working Range

Assay follows the Urea/Ammonia Assay Kit (K-URAMR) standard procedure.
0.1 mL of urea standard was used as sample, with a range of concentrations (0.003-0.140 g/L Urea) which corresponds to 0.3-14 µg of urea per cuvette. Absorbance A2 was taken 5 min after the addition of the 1st trigger enzyme glutamate dehydrogenase (GlDH), giving the measurement of ammonia. Absorbance A3 was taken 5 min after the addition of the final trigger enzyme (urease), giving the measurement of urea. Absorbances were read at 340 nm and 25^{o}C as recommended in the procedure.

For Ammonia analysis 0.1 mL of an ammonia standard was used as sample, with a range of concentrations (0.002-0.07 g/L ammonia) which corresponds to 0.2-7.0 µg of ammonia per cuvette. This standard is not supplied with the Urea/Ammonia Assay Kit (K-URAMR). Absorbance A2 was taken 5 min after the addition of the 1st trigger enzyme GIDH, giving the measurement of ammonia. Absorbance A3 was

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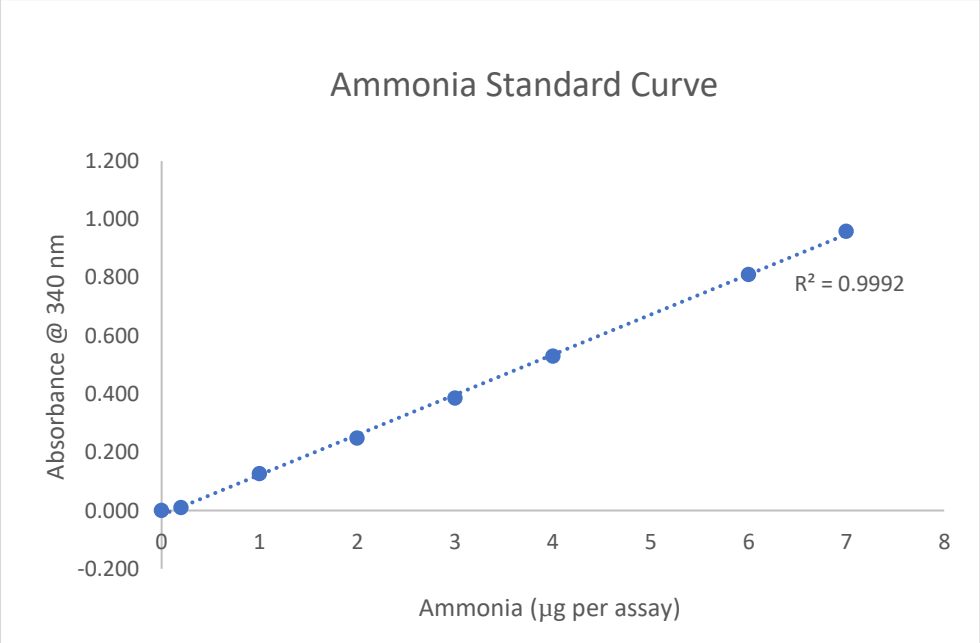
taken 5 min after the addition of the final trigger enzyme (urease), giving the measurement of urea. Absorbances were read at 340 nm and 25°C as recommended in the procedure.

Ammonia Concentration [µg/assay]	**ΔA_{340nm}**
0	0.000
0.2	0.01
1	0.127
2	0.248
3	0.386
4	0.530
6	0.810
7	0.959



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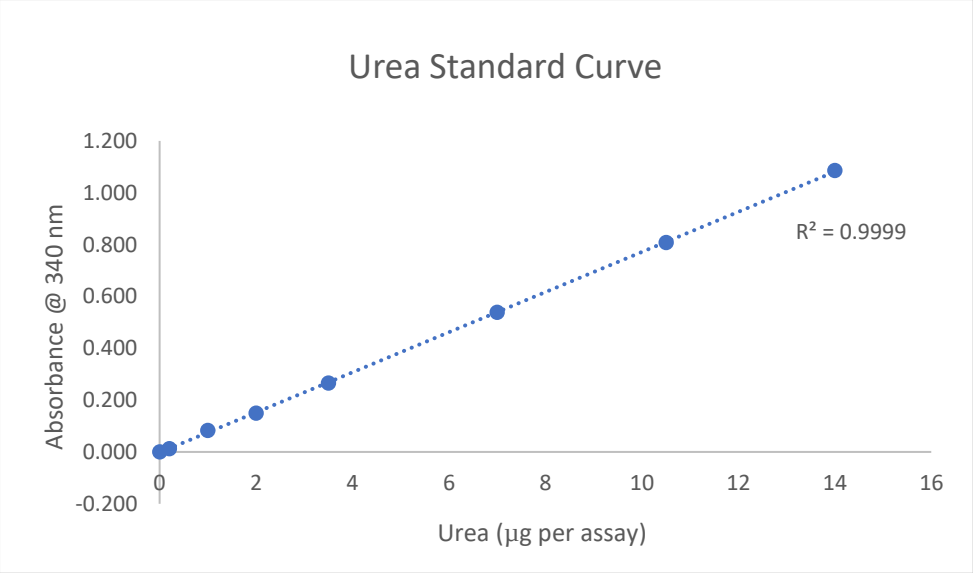
Urea Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.2	0.012
1	0.082
2	0.185
3.5	0.265
7	0.538
10.5	0.807
14	1.085



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.071 mg of ammonia/L and 0.125 mg of urea/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 Urea/Ammonia Assay Kit (K-URAMR) 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 Urea/Ammonia Assay Kit (K-URAMR)

  LOD – For 2.0 mL of sample (maximum volume)

  Ammonia = 0.015 mg/L
  Urea = 0.085 mg/L

  LOQ – For 2.0 mL of sample (maximum volume)
  Ammonia = 0.045mg/L
  Urea = 0.280 mg/L

* **Note:** The above detection limits are for samples as used in the assay, after any required sample preparations (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 Urea/Ammonia Assay Kit (K-URAMR) 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 urea supplied with the Urea/Ammonia Assay Kit (K-URAMR) 0.07 g/L.

Relative Bias *b(%)*

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
Ammonia	22	0.04	0.0396	-1.06
Urea	12	0.07	0.0699	-0.20

3.5. Precision

This report details the reproducibility of the Urea/Ammonia Assay Kit (K-URAMR), 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
Ammonia	22	0.04	0.0396	0.0005	1.29
Urea	12	0.07	0.0699	0.001	1.43

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of ammonia and urea 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	Urea	Ammonia
Working range (µg in cuvette)	0.3-14	0.2-7.0
LOD (mg/L)	0.045	0.015
LOQ (mg/L)	0.280	0.085
Relative Bias *b*(%)	- 0.2	-1.06
Reproducibility (%CV using kit standards)	1.43	1.29