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Validation Report: Primary Amino Nitrogen Assay Kit (PANOPA) (cat. no. K-PANOPA)

1. Scope

Megazyme's Primary Amino Nitrogen Assay Kit (PANOPA) (K-PANOPA) is a UV method used for the measurement and analysis of primary amino nitrogen in grape juice, must and wine. This novel method was developed in-house and measures primary amino acids in mg of N/L.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by the Primary Amino Nitrogen Assay Kit (PANOPA) (K-PANOPA).

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 amino acids containing primary amino groups.
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 isoleucine to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the Primary Amino Nitrogen Assay Kit (PANOPA) (K-PANOPA) standard procedure. 0.05 mL of isoleucine standard was used as sample, with a range of concentrations (4-200 mg of nitrogen/L) which corresponds to 0.2-10.5 µg of nitrogen per cuvette. Absorbance A2 was read after 15 min, at 340 nm and at 25°C as recommended in the procedure.

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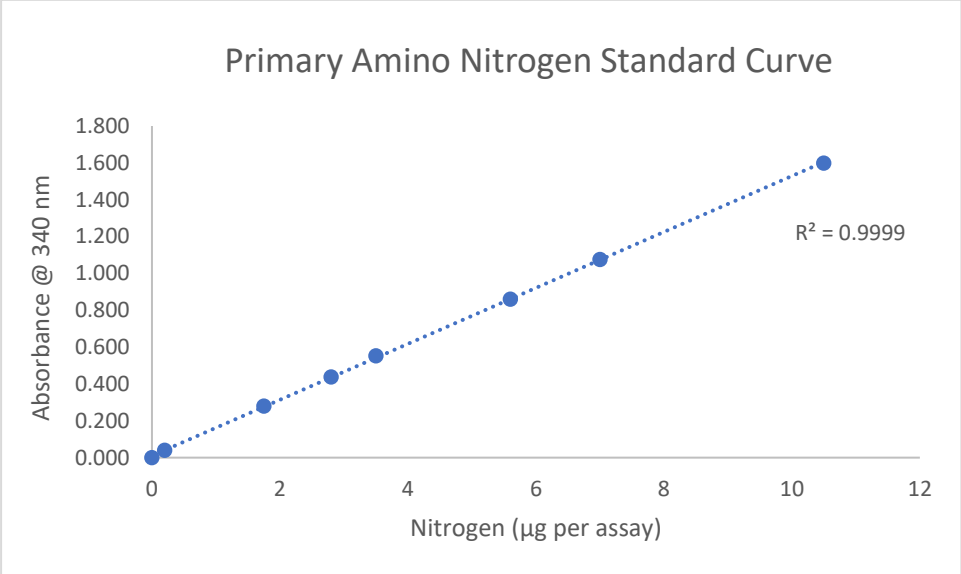
Nitrogen Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.2	0.040
1.75	0.279
2.8	0.437
3.5	0.552
5.6	0.859
7.0	1.076
10.5	1.599



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3.3 LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 2.59 mg/L, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 0.05 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 Primary Amino Nitrogen Assay Kit (PANOPA) (K-PANOPA) 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 Primary Amino Nitrogen Assay Kit (K-PANOPA)

  LOD – For 0.05 mL of sample (maximum volume)
  Isoleucine = 0.389 mg Nitrogen/L

  LOQ – For 0.05 mL of sample (maximum volume)
  Isoleucine = 1.297 mg Nitrogen/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 the Primary Amino Nitrogen Assay Kit (PANOPA) (K-PANOPA) 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 Isoleucine supplied with the Primary Amino Nitrogen Assay Kit (PANOPA) (K-PANOPA) kit at 140 mg of Nitrogen/L.

Relative Bias *b*(%)

	n	Ref Material (mg N/L)	Mean (mg N/L)	*b*(%)
Isoluecine (Nitrogen)	26	140	143.48	2.49

3.5 Precision

This report details the reproducibility of the Primary Amino Nitrogen Assay Kit (PANOPA) (K-PANOPA), 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 (mg N/L)	Mean (mg N/L)	Standard Deviation	%CV
Isoleucine (Nitrogen)	26	140	143.48	1.601	1.12

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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of primary amino nitrogen 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	Nitrogen
Working range (µg in cuvette)	0.2-10.5
LOD (mg Nitrogen/L)	0.389
LOQ (mg Nitrogen/L)	1.297
Relative Bias *b*(%)	2.49
Reproducibility (%CV using isoleucine)	1.12