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Validation Report: Pyruvic Acid Assay Kit (cat. no. K-PYRUV)

1. Scope

Megazyme's Pyruvic acid Assay Kit, (K-PYRUV) is an enzymatic method used for the rapid measurement and analysis of pyruvic acid in beer, wine, fruit juice, food products and bodily fluids. This novel pyruvic acid method was developed in-house and measures pyruvic acid in g/L.

2. Planning

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

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 pyruvic 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 pyruvic acid to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the Pyruvic Acid Assay Kit (K-PYRUV) standard procedure. 0.1 mL of pyruvic acid standard was used as a sample, with a range of concentrations (0.003-0.4 g/L Pyruvic Acid) which corresponds to 0.3-40 µg of pyruvic acid per assay. Absorbance A₂ was read after 3 min, at 340 nm and at 25°C as recommended in the procedure.

The working range is linear between 0.3-40 µg of pyruvic acid per assay.



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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.394 mg/L of pyruvic acid, which is derived from an absorbance difference of 0.020 with a maximum sample volume of 2.0 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 Pyruvic Acid Assay (K-PYRUV).

- The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as $3 \times \sigma$ of the blank sample solution absorbance and $10 \times \sigma$ of the blank sample solution absorbance, respectively, where σ is the standard deviation of the absorbance values from 10 replicates.
- For Pyruvic Acid Assay Kit (K-PYRUV)

LOD – For 2.0 mL of sample (maximum volume)

Pyruvic Acid = 0.039 mg/L

LOQ – For 2.0 mL of sample (maximum volume)

Pyruvic Acid = 0.138 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.



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3.4. Trueness (*Bias*)

Comparison of the mean of the results (x) achieved with the Pyruvic Acid Assay Kit (K-PYRUV) with a suitable reference value (x_{ref}). For this report, Relative Bias is calculated in per cent as: $b(\%) = x - x_{ref} / x_{ref} \times 100$. The reference material for this purpose is pyruvic acid supplied with the Pyruvic Acid Assay Kit (K-PYRUV) at 0.2 g/L.

Relative Bias $b(\%)$

| | n | Ref Material (g/L) | Mean (g/L) | $b(\%)$ |
|--------------|----|--------------------|------------|---------|
| Pyruvic Acid | 26 | 0.2 | 0.1982 | -0.92 |

3.5. Precision

This report details the reproducibility of the Pyruvic acid Assay Kit (K-PYRUV), 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 |
|--------------|----|--------------------|------------|--------------------|------|
| Pyruvic Acid | 26 | 0.2 | 0.1982 | 0.0030 | 1.51 |



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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of pyruvic acid in various matrices. It is a novel method 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 | Pyruvic Acid |
|---|--------------|
| Working range (μg in cuvette) | 0.3 - 40 |
| LOD (mg/L) | 0.039 |
| LOQ (mg/L) | 0.138 |
| Relative Bias <i>b</i> (%) | -0.92 |
| Reproducibility (%CV using kit standard) | 1.51 |