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Validation Report: Ethanol Assay Kit (Liquid Ready Reagents) **(cat. no. K-ETOHLQR)**

1. Scope

Megazyme's Ethanol Assay Kit (K-ETOHLQR) is an enzymatic method used for the rapid measurement and analysis of Ethanol in foodstuffs, beverages and other materials. This method was developed in-house and measures ethanol in g/L or % (v/v).

2. Planning

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

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 ethanol.

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 ethanol to the sample in the initial extraction steps.

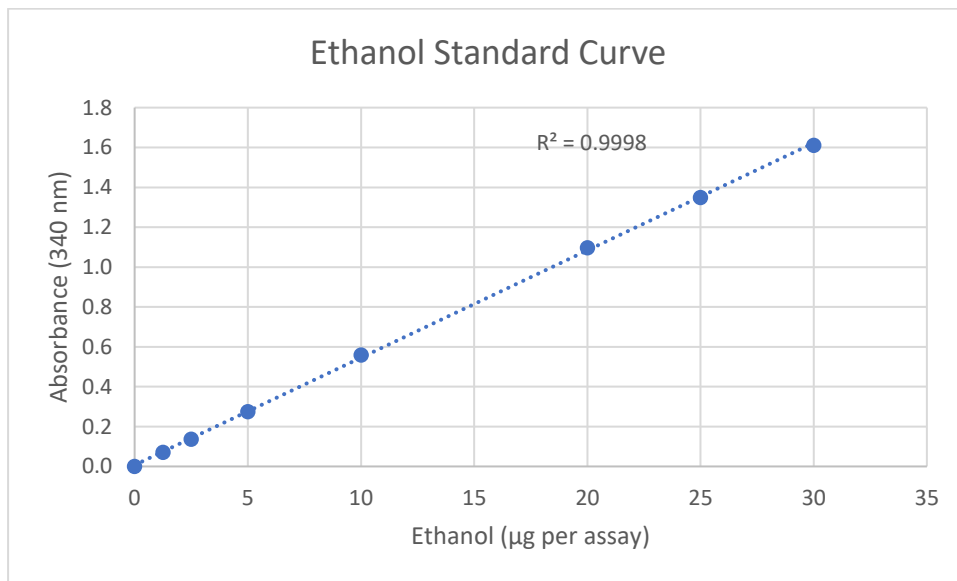
3.2. Working Range

Assay follows the K-ETOHLQR standard manual assay procedure. 0.1 mL of Ethanol standard was used as sample, with a range of concentrations (0.0125-0.3 g/L ethanol) which corresponds to 1.25-30 µg of ethanol per cuvette. Absorbance A₂ was read after 7 min, at 340 nm and at 25°C as recommended in the procedure.



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Ethanol Concentration [$\mu\text{g}/\text{cuvette}$]	$\Delta A_{340\text{nm}}$
0	0
1.25	0.0716
2.5	0.1377
5	0.2757
10	0.5596
20	1.098
25	1.3497
30	1.6119





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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 3.8 mg/L, which is derived from an absorbance difference of 0.020 with the maximum sample volume of 0.1 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 Ethanol Assay Kit (K-ETOH) measurement procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as $3 \times s'0$; where $s'0$ is the standard deviation of the absorbance change for replicate determinations of a low concentration reference sample.
- The LOQ is the lowest level at which the kit's performance is acceptably repeatable. LOQ is calculated as $kQ \times s'0$; where $s'0$ is the standard deviation of the absorbance change for replicate determinations of a low concentration reference sample. The IUPAC default value for kQ is 10.
- For Ethanol Assay Kit (K-ETOH)

LOD – For 0.1 mL of sample volume

Ethanol = 1.6 mg/L

LOQ – For 0.1 mL of sample volume

Ethanol = 5.3 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 Ethanol Assay Kit (K-ETOHLQR) method 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 ethanol supplied with the Ethanol Assay Kit (K-ETOHLQR) at concentrations across the working range (0.0125-0.3 g/L ethanol).

Relative Bias $b(\%)$

	n	Ref Material (g/L)	Mean (g/L)	$b(\%)$
Ethanol	10	0.0125	0.014	7.43
	6	0.025	0.025	4.10
	6	0.05	0.052	3.77
	6	0.1	0.104	4.40
	6	0.2	0.205	2.75
	6	0.25	0.252	0.73
	6	0.3	0.299	-0.46
Mean				3.24

3.5 Precision

This report details the reproducibility of the Ethanol Assay Kit (K-ETOHLQR), 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

	Ref Material (g/L)	n	Mean (g/L)	Standard Deviation	%CV
Ethanol	0.0125	6	0.013	0.0003	2.00
	0.025	6	0.025	0.0005	2.06
	0.05	6	0.052	0.0015	2.86
	0.1	6	0.104	0.0017	1.59
	0.2	6	0.205	0.0034	1.64
	0.25	6	0.252	0.0043	1.69
	0.3	6	0.299	0.0072	2.41
Mean				0.003	2.036



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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of ethanol 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	Ethanol
Working range (μg in cuvette)	1.25-30
LOD (mg/L)	1.6
LOQ (mg/L)	5.3
Relative Bias <i>b</i> (%)	3.24
Reproducibility (%CV using kit standard)	2.036