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Validation Report: D-/L-Lactic Acid (D-/L-Lactate) (Rapid) Assay Kit (cat. no. K-DLATE)

1. Scope

Megazyme's D-/L-Lactic Acid Assay Kit (K-DLATE) is an enzymatic method used for the rapid and specific concurrent measurement and analysis of D-lactic acid and L-lactic acid in beverages, meat, dairy and food products. This method was developed in-house and measures D-lactic acid and L-lactic acid in g/L. Methods based on this principle have been accepted by DIN, GOST, IDF, EEC, EN, ISO, OIV, IFU, AIJN and MEBAK.

2. Planning

The purpose of this report is to verify and validate the current method as detailed by D-/L-Lactic Acid Assay Kit (K-DLATE).

3. Performance characteristics

The selectivity, working range, limit of detection, limit of quantification, trueness (*bias*) and precision of this kit will be detailed in this report.

3.1. Selectivity

This assay is specific for either D-lactic acid or L-lactic acid. The assay can be used to measure D- or L-lactic acid separately, sequentially, or for total lactic 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 D-/L-lactic acid to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the D-/L-Lactic Acid Assay Kit (K-DLATE) individual acid procedure for each acid.

For D-lactic acid, 0.1mL of D-lactic acid standard was used as sample, with a range of concentrations (0.005-0.3 g/L D-lactic acid) which corresponds to 0.5-30 µg of D-lactic acid per cuvette. Absorbance A2 was read after 5 min after the addition of the trigger enzyme, (D-LDH) giving the concentration of D-lactic acid in the cuvette.

For L-lactic acid, 0.1mL of L-lactic acid standard was used as sample, with a range of concentrations (0.005-0.3 g/L L-lactic acid) which corresponds to 0.5-30 µg of L-lactic acid per cuvette. Absorbance A2 was read after 10 min after the addition of the trigger enzyme, (L-LDH) giving the concentration of L-lactic acid in the cuvette.

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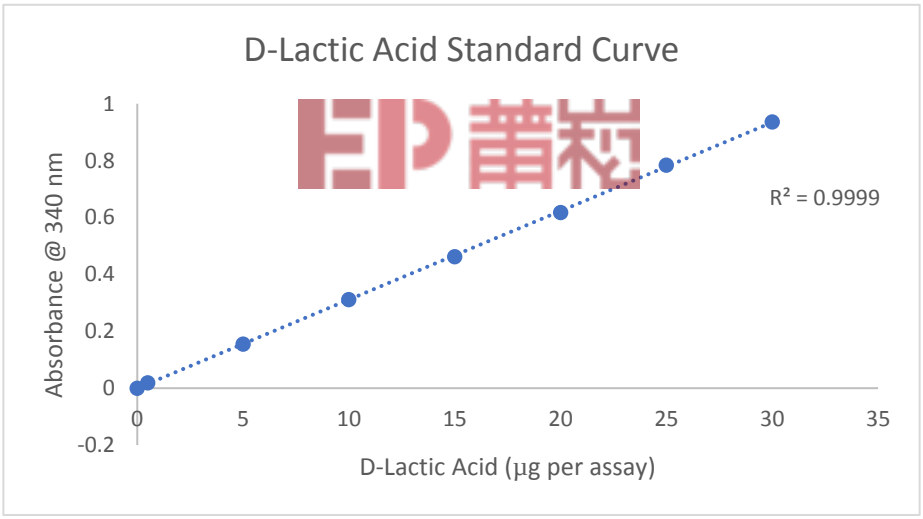
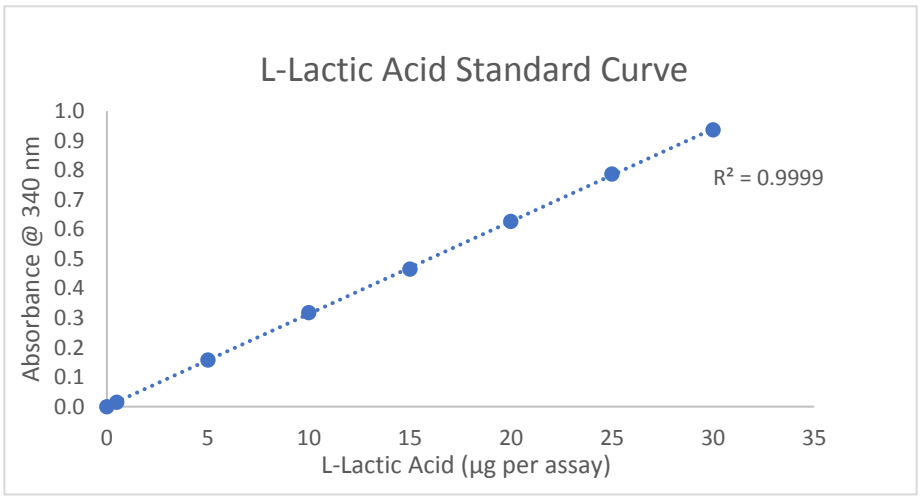
All absorbances were read at 340 nm and 25°C as recommended in the procedure.

D-Lactic Acid Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.5	0.019
5	0.155
10	0.312
15	0.462
20	0.618
25	0.784
30	0.936

L-Lactic Acid Concentration [µg/assay]	ΔA_{340nm}
0	0.000
0.5	0.015
5	0.158
10	0.318
15	0.466
20	0.627
25	0.787
30	0.937





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3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, 0.214 mg/L of either D/L-lactic acids, which is derived from an absorbance difference of 0.010 and the maximum sample volume of 1.50 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 D-/L-Lactic Acid Assay Kit (K-DLATE) measurement 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 D-/L-Lactic Acid Assay Kit (K-DLATE)

  LOD – For 1.5 mL of sample (maximum volume)
  D-Lactic Acid = 0.133 mg/L
  L-Lactic Acid = 0.067 mg/L

  LOQ – For 1.5 mL of sample (maximum volume)
  D-Lactic Acid = 0.4 mg/L
  L-Lactic Acid = 0.267 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 D-/L-Lactic Acid Assay Kit (K-DLATE) 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 D-lactic acid and L-lactic acid supplied with the D-/L-Lactic Acid Assay Kit (K-DLATE) at 0.15 g/L each.

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	*b*(%)
D-Lactic Acid	18	0.15	0.1524	1.61
L-Lactic Acid	18	0.15	0.1525	1.14

3.5. Precision

This report details the reproducibility of the D-/L-Lactic Acid Assay Kit (K-DLATE), 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
D-Lactic Acid	18	0.15	0.1525	0.0019	1.24
L-Lactic Acid	18	0.15	0.1524	0.0013	0.83

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Repeatability of this kit can be assessed using wine samples. This is a measure of the variability in results by a single analyst, using real samples, using the same equipment and over a short period of time. The use of wine samples shows one of the many applications of this kit.

Repeatability

D-Lactic Acid	n	Mean (g/L)	Standard Deviation	%CV
White Wine	12	0.1	0.002	1.601
Red Wine	12	0.366	0.008	2.172

L-Lactic Acid	n	Mean (g/L)	Standard Deviation	%CV
White Wine	12	0.007	0.001	7.563
Red Wine	12	1.677	0.030	1.781

4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of D-/L-lactic acid 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.

K-DLATE Validation Summary	D-Lactic Acid	L-Lactic Acid
Working range (µg in cuvette)	0.3-30	0.3-30
LOD (mg/L)	0.133	0.067
LOQ (mg/L)	0.4	0.267
Relative Bias *b* (%)	1.61	1.64
Reproducibility (%CV using kit standard)	0.825	1.238
Repeatability (%CV using white wine)	1.601	7.563
Repeatability (%CV using red wine)	2.172	1.781