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Validation Report: Succinic Acid Assay Kit (cat. no. K-SUCC)

1. Scope

Megazyme's Succinic Acid Assay Kit (K-SUCC) is an enzymatic method used for the rapid measurement and analysis of succinic acid in wine, foodstuffs, and other materials. This succinic acid method was developed in-house and measures succinic acid in g/L. Methods based on this principle have been accepted by EEC.

2. Planning

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

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

Other than succinic acid, succinyl-CoA synthetase also reacts with itaconic acid. However, the level of itaconic acid in foodstuffs is so low that it does not interfere with the analytical results obtained with this kit.

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 succinic acid to the sample in the initial extraction steps.

3.2. Working Range

Assay follows the Succinic Acid Assay Kit (K-SUCC) standard procedure. Succinic acid standard was used as a sample across a range of concentrations (0.008-0.4 g/L succinic acid) which corresponds to 0.8-40 µg of succinic acid per cuvette.

The working range is linear between 0.8-40 µg of succinic acid per assay.



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3.3. LOD and LOQ Range

The **instrument limit of detection** for the standard manual assay procedure is 0.256 mg/L, which is derived from an absorbance difference of 0.01 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 Succinic Acid Assay Kit (K-SUCC) 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 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 $k_Q \times s'_0$; where s'_0 is the standard deviation of a number of samples A1 reading. The IUPAC default value for k_Q is 10.
- For Succinic Acid Assay Kit (K-SUCC)

LOD – For 2.0 mL of sample (maximum volume)
Succinic Acid = 0.154 mg/L

LOQ – For 2.0 mL of sample (maximum volume)
Succinic Acid = 0.539 mg/L

* **Note:** The above detection limits are for samples as used in the assay after sample preparation, if required (e.g. deproteinisation). The dilution used in pre-treatment must be accounted for while establishing the detection limits for specific samples.

3.4. Trueness (*Bias*)

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

Relative Bias *b*(%)

	n	Ref Material (g/L)	Mean (g/L)	<i>b</i> (%)
Succinic Acid	19	0.2	0.1972	-1.39

3.5. Precision

This report details the reproducibility of the Succinic Acid Assay Kit (K-SUCC), 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
Succinic Acid	19	0.2	0.1972	0.0011	0.58

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

	n	Mean (g/L)	Standard Deviation	%CV
White Wine	12	0.417	0.008	1.86
Red Wine	12	0.691	0.011	1.58



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4. Conclusion

The method outlined in this document is a robust, quick and easy method for the measurement of Succinic 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.

Validation Summary	Succinic Acid
Working range (μg in cuvette)	0.8-40
LOD (mg/L)	0.154
LOQ (mg/L)	0.539
Relative Bias <i>b</i> (%)	-1.39
Reproducibility (%CV using kit standard)	0.58
Repeatability (%CV using white wine)	1.86
Repeatability (%CV using red wine)	1.58