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## **Validation report: Formic Acid Assay kit (cat. no. K-FORM)**

### **1. Scope**

Megazyme's Formic Acid Assay kit (K-FORM) is an enzymatic method used for the rapid measurement and analysis of Formic Acid in foodstuffs, beverages and other materials. This method was developed in-house and measures Formic Acid in g/L. Methods based on this principle have been accepted by MEBAK.

### **2. Planning**

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

### **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 formic 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 formic acid to the sample in the initial extraction steps.

#### **3.2. Working Range**

Assay follows the Formic Acid Assay Kit (K-FORM) standard procedure. 0.1mL of Formic Acid standard was used as sample, with a range of concentrations (0.04-0.20 g/L formic acid) which corresponds to 0.4-20 µg of formic acid per assay. Absorbance A<sub>2</sub> was read after 12 min at 340nm and at 25°C as recommended in the standard assay procedure.

The working range is linear between 0-20 µg of formic acid per assay.



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### 3.3. LOD and LOQ

The **instrument limit of detection**, as per kit booklet, is 0.0932 mg/L, which is derived from an absorbance difference of 0.010 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 Formic Acid Assay Kit (K-FORM) measurement procedure.

- The LOD is the lowest concentration of the analyte that can be detected by the method. LOD is calculated as  $3 \times s'_o$ ; where  $s'_o$  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  $k_Q \times s'_o$ ; where  $s'_o$  is the standard deviation of a number of samples A1 reading. The IUPAC default value for  $k_Q$  is 10.
- For Formic Acid Assay Kit (K-FORM)

**LOD – For 2.0 mL of sample (maximum volume)**

Formic Acid = 0.317 mg/L

**LOQ – For 2.0 mL of sample (maximum volume)**

Formic Acid = 1.062 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.



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### 3.4. Trueness (Bias)

Comparison of the mean of the results ( $\bar{x}$ ) achieved with Formic Acid Assay Kit (K-FORM) method with a suitable reference value ( $\bar{x}_{ref}$ ). For this report, Relative Bias is calculated in per cent as:  $b(\%) = \frac{\bar{x} - \bar{x}_{ref}}{\bar{x}_{ref}} \times 100$ . The reference material for this purpose is formic acid, supplied with the Formic Acid Assay Kit (K-FORM), and detailed to be prepared at 0.1 g/L in the kit data booklet.

#### Relative Bias $b(\%)$

	n	Ref Material (g/L)	Mean (g/L)	$b(\%)$
Formic Acid	20	0.1	0.0970	-3.00

### 3.5. Precision

This report details the reproducibility of the Formic Acid Assay Kit (K-FORM), 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 are used as the reference material.

#### Reproducibility

	n	Ref Material (g/L)	Mean (g/L)	Standard Deviation	%CV
Formic Acid	20	0.1	0.0970	0.0012	1.19



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**4. Conclusion**

The method outlined in this document is a robust, quick and easy method for the measurement of Formic 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	Formic Acid
Working range ( $\mu\text{g}$ in cuvette)	0.4-20
LOD (mg/L)	0.317
LOQ (mg/L)	1.062
Relative Bias <i>b</i> (%)	-3.00
Reproducibility (%CV using kit standard)	1.19

