UV assay for the determination of formic acid in foodstuffs and other sample materials
Test combination for 25 determinations

For *in vitro* use only
Store between 2 - 8 °C

1. Test principle

Formic acid (formate) is quantitatively oxidized to bicarbonate (CO_2) by nicotinamide-adenine-dinucleotide (NAD) in presence of formate dehydrogenase (FDH):

$$\text{Formic acid} + NAD^+ + H_2O \xrightarrow{FDH} CO_2 + NADH + H^+$$

In this process, nicotinamide-adenine-dinucleotide (NAD) is reduced to NADH. The increase in NADH is measured based on its specific absorption at a wavelength of 340 nm. The result is expressed as formic acid [g/L].

2. Reagents

2.1. Content & composition

The test is suitable for manual and automated processing. With manual processing, the reagents are sufficient for 25 determinations. The number of determinations for automated processing is increased by a multiple; however it depends on the device.

- Reagent 1: 1 x 50 mL with buffer, FDH
- Reagent 2: 1 x 12.5 mL with buffer, NAD

2.2. Reagent preparation

The reagents are ready-to-use and be allowed to reach room temperature (20 - 25 °C) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

The reagents are stable until the end of the month of the indicated shelf life (see label) even after opening at 2 - 8 °C if handled properly. Do not freeze reagents.

2.4. Safety & disposal

The general safety rules for working in chemical laboratories should be applied. Do not swallow! Avoid contact with skin and mucous membranes.

This kit may contain hazardous substances. For hazard notes on the contained substances, please refer to the appropriate safety data sheets (SDS) for this product. After use, the reagents can be disposed of with the laboratory waste. Packaging materials may be recycled.

3. Sample preparation

- Sample preparation for manual and automated testing is identical.
- The samples should be brought to room temperature before measurement.
- Use liquid, clear and almost neutral sample solutions directly or after dilution with dist. water. Water to a concentration within the measuring range (see performance data).
- Filter or centrifuge turbid solutions.
- If necessary, decolorize strongly colored samples.
- Degas samples containing carbonic acid.
- Crush or homogenize solid or semi-solid samples. Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water; filtrate or clarify if necessary.
- If necessary, clarify protein-containing samples with Carrez reagents.

4. Assays performance

Wavelength: 340 nm
Temperature: 20 - 37 °C (during the measurement)
Measurement: against air (without cuvette) or water
Measuring range: 5 - 400 mg/L

	Reagent blank	Sample / control
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for 3 min at 20 - 37 °C. Read absorbance A_1, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for 40 min at 20 - 37 °C and read absorbance A_2.		

If the formic acid concentration is below 50 mg/L, the sample volume must be increased to 200 µL; the same applies to the reagent blank value in this case.

The reagent blank value must be determined once for each run and subtracted from each sample result.

5. Calculation of results

5.1. Calculation of sample solutions

$$\Delta A = (A_2 - df \times A_1)_{sample} - (A_2 - df \times A_1)_{RB}$$

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume + R1}}{\text{test volume}} = 0.808$$

Increasing the sample volume (up to max. 1000 µL) with unchanged reagent volumes requires conversion of the reagent dilution factor (df). If the volume of very acidic samples is increased, the test system may be affected. This must be checked.

$$\mathbf{C_{formic\ acid}\ [g/L]} = \frac{(V \times MW \times \Delta A)}{(\varepsilon \times d \times v \times 1000)} = \mathbf{0.190 \times \Delta A}$$

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight [g/mol]	= 46.03
d:	Optical path [cm]	= 1.00
v:	Sample volume [mL]	= 0.100
ε:	Extinction coefficient NADH [L/mmol x cm]	= 6.3 (at 340 nm)

5.2. Calculation of solid samples

$$\text{Content}_{\text{formic acid}}\ [g/100\ g] = \frac{C_{\text{formic acid}}\ [g/L\ \text{sample solution}]}{\text{weight}_{\text{sample}}\ \text{in g/L sample solution}} \times 100$$

5.3. Controls & acceptance criteria

Controls or reference samples should be carried along for quality control during each run. The recovery of formic acid control solutions should be within 100 ± 5 %. The recovery for extracted food samples should be within 100 ± 10 %.

Note: It is generally recommended to test samples quickly after opening and extraction.
When analyzing the pure substance formic acid, results of less than 100 % can be expected, since the pure acid gradually decomposes into CO and water. (When preparing the formic acid solution, the volatility of the formic acid must be taken into account.)

6. Performance data

6.1. Specificity & side activities

The formate dehydrogenase is specific to formic acid. Other carboxylic acids such as acetic acid, oxalic acid or citric acid do not affect the test.

6.2. Interferences

Formaldehyde and histamine do not interfere up to 10 g/L and hydrogen peroxide and ascorbic acid up to 5 g/L. For sodium sulphite, a lower recovery can be expected from a concentration of 1.5 g/L.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 500 mg/L formic acid, with the recommended measuring range between 5 and 400 mg/L (sample volume of 100 µL).
The limit of detection (LoD) was determined for a sample volume of v = 100 µL according to method DIN 32645:2008-11, using aqueous formic acid solution. This results in an LoD of 0.5 mg/L.
The limit of quantification (LoQ) was determined by precision profile (200 µL sample volume) and is 2.5 mg/L.
The smallest absorbance difference that the method can distinguish is ΔA = 0.005. For a sample volume of v = 1000 µL, this results in an LoD of 0.128 mg/L. Based on ΔA = 0.010, an LoQ of 0.256 mg/L was calculated.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Validation reports
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Excel templates for results calculation
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) und certificates of analysis (CoA) are available in digital form under the following link
https://eifu.r-biopharm.com/

8. Services & technical support

On request, we offer the following services:

- Customized troubleshooting
- Data & results analysis
- Customer workshops & webinars
- Automation: application support and technical service

9. Disclaimer

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