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 (36 – 46 °F)

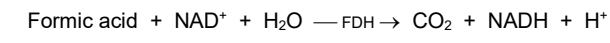
This test was validated using selected samples of the following matrices: wine, vinegar, sauerkraut, fruit juices, honey, jam, and tomato paste.

Detailed results and information regarding associated validation data are found in the Validation Report.

The test may be used with other foods or samples material, provided that these are subjected to individual validation by the user.

1. Test principle

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



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 in 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 / 68 – 77 °F) before use. Do not interchange components between kits of different batches.

2.3. Storage & stability

If stored as directed and between 2 – 8 °C (36 – 46 °F), reagents remain stable until the printed expiration date, even after opening. Reagents must not be frozen.

2.4. Safety & disposal

The test is intended solely for the intended use as described. The provided Instructions for Use must be strictly followed.

Follow standard chemical safety procedures when handling this product. Do not swallow. Avoid contact with skin or mucous membranes.

Detail safety information for individual components is available in the corresponding Safety Data Sheets (SDS).

Dispose of used reagents as laboratory waste in compliance with all relevant regulations. Packaging materials are to be recycled according to local regulations.

3. Sample preparation

3.1. General

- Sample preparation for manual and automated testing is the same.
- Bring sample solutions to room temperature before measurement.
- Use liquid, clear, colorless and almost neutral sample solutions directly or dilute sufficiently to yield a formic acid concentration within the stated measuring range (refer to performance data).
- In case of higher sample volumes (up to 1000 µL), check the pH value of the test solution and neutralize in case of any doubt.
- Adjust acidic or weakly colored samples to pH 7 – 8 by adding sodium or potassium hydroxide solution and incubate for 15 minutes. If necessary, measure against a sample blank.
- Treat strongly colored samples with activated charcoal or PVPP (e.g. 2 g/100 mL) and filter.
- For turbid test samples: Filter by using fluted paper filter or syringe filter or centrifuge the test solution in a reaction tube (recommended 3000 rpm for at least 5 minutes) until a clear filtrate or supernatant is obtained.
- Degas samples containing carbonic acid, e.g. by stirring in a beaker, applying a brief ultrasonic burst, filtration or centrifugation.
- Crush and homogenize solid and semi-solid samples. Weigh a sufficient quantity of sample in a volumetric flask (considering the measuring range), extract with water; fill up to the mark and filter if necessary (by using fluted paper or syringe filters) or centrifuge in reaction tubes. Use Carrez clarification if necessary.
- Fat containing samples: weigh a sufficient quantity (considering the measuring range) into a volumetric flask and extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 minutes and filter.
- Deproteinize samples containing proteins with perchloric acid or with trichloroacetic acid.
- Alternatively, clarify samples containing proteins or fat with Carrez reagents:
  Weigh an appropriate sample quantity accurately into a 100 mL volumetric flask and add approx. 60 mL distilled water. In case of liquid samples, pipette the sample into a 100 mL volumetric flask or beaker pre-filled with 60 mL distilled water. Add 5 mL Carrez I solution (3.60 g potassium hexacyano-ferrate(II)-trihydrate $K_4[Fe(CN)_6]$ x 3 H_2O/100 mL) and 5 mL Carrez II solution (7.20 g zinc sulfate $ZnSO_4$ x 7 H_2O/100 mL). Mix well after each addition. Adjust the pH with 0.1 M NaOH to a value between 7.5 and 8.5. Transfer into a 100 mL volumetric flask, fill up to the mark, mix and filter using fluted paper filters or syringe filters.

3.2. Determination of formic acid in tomato paste or tomato ketchup (§ 64 method 26.11.03-15)

- Weigh 10 g ± 0.010 g of the sample into a beaker (150 mL) and add 50 mL distilled water.
- Homogenize the mixture for 10 minutes on a magnetic stirrer. Transfer the suspension quantitatively into a volumetric flask (100 mL), fill up to the mark with distilled water and mix.
- Transfer 10 mL of this suspension into a volumetric flask (50 mL) and fill up to the mark with distilled water.
- Filter the slurry through a pleated filter into a flask, discarding the first 15 mL. Optionally, the slurry can also be centrifuged.
- 500 µL of the solution obtained is used in the test.

3.3. Determination of formic acid in mixed pickles

- Separate the liquid from the solid phase, e.g. by filtration.
- For fat separation, place the sample for 30 minutes in a refrigerator or for 20 minutes in an ice-bath, filter and dilute if necessary.

3.4. Determination of formic acid in fruit juices

- Use light colored fruit juices directly or diluted with distilled water.
- When analyzing strongly colored fruit juices, 100 mg charcoal or PVPP are added to 5 mL juice.
- Mix for 1 minute and filter with a sterile syringe filter (0.22 µm).
- Adjust strong acidic juices to pH 7 – 8 with NaOH or KOH (1 M or 5 M).

Note: juices which are not a clear solution after treatment may result in higher variations or discrepancies in the determination process, e.g. orange juice.

3.5. Determination of formic acid in wine

- Wine samples should be treated in the same way as fruit juices.
- When analyzing red wine, add 100 mg charcoal to 5 mL sample, mix for 1 minute and filter trough a sterile syringe filter (0.22 µm).
- Use 200 µL for the assay.

3.6. Determination of formic acid in vinegar

- Neutralize samples with NaOH or KOH (1 M or 5 M) to pH 7 – 8. Dilute with distilled water at a ratio of 1:1.
- Use 200 µL for the assay.

3.7. Determination of formic acid in jam

- Weight 20 g of a homogenized sample into a 100 mL volumetric flask and add 20 mL hot distilled Water (60 °C / 140 °F).
- Subsequently, add 2 mL Carrez I solution (15 g potassium hexacyano-ferrate(II)-trihydrate $K_4[Fe(CN)_6]$ x 3 H_2O/100 mL) and 2 mL Carrez II solution (30 g zinc sulfate $ZnSO_4$ x 7 H_2O/100 mL) into the flask.
- Adjust the pH to approx. 7.5 – 8.5 with around 3 mL NaOH (1 M). Mix after each addition. Fill up to the mark with distilled water, mix again and filter.
- Use 500 µL of the filtrate for the determination.

4. Manual test procedure

Wavelength:	340 nm
Temperature (measurement):	20 – 37 °C (68 – 99 °F)
Photometer alignment:	against air (without cuvette)
Measuring range:	5 – 400 mg/L

	Reagent blank	**Samples / controls**
Reagent 1	2000 µL	2000 µL
Sample / control	-	100 µL
Dist. water	100 µL	-
Mix, incubate for **3 minutes at 20 – 37 °C (68 – 99 °F)**. **Read** absorbance **A_1**, then addition of:		
Reagent 2	500 µL	500 µL
Mix, incubate for **40 minutes at 20 – 37 °C (68 – 99 °F)** and **read** absorbance **A_2**.		

Important notes for assay procedure

- The reagent blank value (water sample) must be determined **in each series of measurement** and subtracted from **each** sample result.
- Specified incubation times were validated at 37 °C (99 °F). The test may generally perform within a range between **20 – 37 °C (68 – 99 °F)**.
- 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.
- Use separate tips for each sample extract and the control solutions to avoid cross-contamination; rinse the tip before pipetting.
- A multistepper pipette is recommended for adding reagents. Use a separate tip for each component.
- Stirring spatulas are recommended for mixing each individual cuvette. Remove these from the cuvette immediately before measuring the absorbance
- Always wait for the reaction to end or for the absorbance to stabilize (at least during the first test runs or validation). If the absorbance has not stopped after the recommended incubation time, continue measuring at 2- or 5-minute intervals, for example, until a constant absorbance value is reached.
- If a creep reaction occurs, the reaction will not have finished after stated incubation times and will typically show a constant increase of ΔA. Calculate the analyte-specific ΔA value by plotting the absorbance values against time and performing a linear regression to determine the rate of increase in ΔA per minute related to the creep reaction. Then, extrapolate the absorbance to the time at which reagent 2 is added.
- If the measured absorbance difference of the samples is too small (< 0.020), the sample solution must be prepared again with a higher weight or a lower dilution.
- If the absorbance difference of the samples is very large (e.g., > 1.500), the sample solution must be diluted if necessary.

5. Calculation of results

5.1. Concentration of formic acid in sample solutions

The extinction difference ΔA must be calculated for each sample:

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

df: Dilution factor
RB: Reagent blank

$$df = \frac{\text{sample volume [mL]} + \text{volume R1 [mL]}}{\text{reaction volume [mL]}} = 0.808$$

The specified df value of **0.808 applies to a base application of 100 µL**. An increase in sample volume is possible (max. 1000 µL; refer to validation report). **While keeping reagent volumes unchanged**, this requires **conversion of the reagent dilution factor (df) accordingly**.

Increasing the sample volume may influence test performance. This must generally be checked depending on the matrix. **The reagent blank value must be adjusted to the changed sample volume.**

The concentration of formic acid in sample solutions is calculated using Lambert-Beer's law:

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

If the sample solution was diluted before measurement, this result has to be multiplied with the **sample pre-dilution factor F**.

V:	Test volume basic application [mL]	= 2.600
MW:	Molecular weight formic acid [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. Content of formic acid in solid samples

When analyzing solid and semi-solid samples that have to be weighed in for the extraction of the sample, the content is related to the sample weight:

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

5.3. Controls & acceptance criteria

Control or reference samples should be included in each run for quality control purposes. Therefore, we recommend Enzytec™ *Liquid* Multi-Acid Standard 2 *low* (Art. No. E8470; with 0.250 g/L formic acid).

The recovery of this standard and other *aqueous* control solutions should be 100 ± 5 %, for extracted food samples, it should be within the range of 90 – 110 %.

Note: It is generally recommended to analyze samples quickly after opening or after 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, ascorbic acid, citric acid, D-lactic acid, L-lactic acid, D-malic acid, L-malic acid, gluconic acid, glycolic acid, propionic acid and tartaric acid showed no side activity.

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 sulfite, the endpoint of the reaction is expected to be reached with a slight delay at a concentration of approx. 0.8 g/L, higher concentrations may lead to lower recoveries within the stated incubation time.

6.3. Linearity, measuring range & sensitivity

Linearity is given up to 500 mg/L formic acid (100 µL sample volume) with a recommended measuring range of 5 – 400 mg/L.

The limit of detection (LoD) was determined according to the method DIN 32645:2008-11 in a buffered aqueous solution. For a sample volume of 100 µL, the calculated LoD is 1.0 mg/L and 0.1 mg/L for a sample volume of 1000 µL.

The limit of quantification (LoQ) was determined by precision profile. The calculated LoQ is 2.0 mg/L formic acid for a sample volume of 100 µL and 0.2 mg/L for a sample volume of 1000 µL.

7. Supporting documents

On request, we offer the following documents:

- Enzytec™ *Liquid* Formic acid Validation reports
- Enzytec™ *Liquid* Formic acid Excel template for results
- Enzytec™ *Liquid* Formic acid Technical Information
- Enzytec™ *Liquid* Sample preparation guide
- Enzytec™ *Liquid* Troubleshooting guide

Safety data sheets (SDS) and certificates of analysis (CoA) are available in digital form, quoting the batch number, via the following link:

https://eifu.r-biopharm.com/

8. Limits of this method

Test results may vary depending on the sample matrix, specific test implementation, and laboratory environmental conditions. Detection and quantification limits are dependent on respective sample matrices extraction procedures. Refer toto the current Validation Report for details.

For this test, only the matrices explicitly listed in the documentation were validated, due to the wide variety of food products and other potential sample materials.

When analysing non-validated matrices results should be verified by performing spiking (fortification) experiments. If appropriate or necessary, a suitable sample preparation procedure for the respective matrix must be developed and validated.

The responsibility for validating non-validated matrices and for ensuring the suitability of the assay for its intended use lies solely with the user.

9. Services & technical support

On request, we offer the following services:

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

10. Disclaimer

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d. Failure to otherwise use, and when necessary validate or verify, suitable controls, samples, matrices, or processing procedures;
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