UV-method
for the determination of formic acid in foodstuffs and other materials
Cat. No. 10 979 732 035
Test-Combination for 21 determinations

Principle (Lit. 1)
Formic acid (formate) is quantitatively oxidized to bicarbonate by nicotinamide-adenine dinucleotide (NAD) in the presence of formate dehydrogenase (FDH).

\[
\text{FDH} \quad \text{NAD} + \text{HCO}_2^- \rightarrow \text{NADH} + \text{HCO}_3^-
\]

The amount of NADH formed is stoichiometric to the amount of formic acid. The increase in NADH is measured by means of its light absorbance at 334, 340 or 365 nm.

The Test-Combination contains
1. Bottle 1 with approx. 22 ml solution, consisting of:
   - potassium phosphate buffer, pH approx. 7.5
2. Bottle 2 with approx. 420 mg NAD, Li salt, lyophilizate
3. Bottle 3 with formate dehydrogenase lyophilizate, approx. 80 U

Preparation of solutions
1. Dissolve contents of bottle 2 with the contents of bottle 1 using a magnetic stirrer, if necessary (= reaction mixture 2).
2. Dissolve contents of bottle 3 with 12 ml redist. water (= solution 3).

Stability of reagents
Solution 1 is stable at 2-8°C (see pack label).
Bring solution 1 to 20-25°C before use.
The contents of bottle 2 are stable at 2-8°C (see pack label).
Reaction mixture 2 is stable for 2 weeks at 2-8°C.
Bring reaction mixture 2 to 20-25°C before use.
The contents of bottle 3 are stable at 2-8°C (see pack label).
Solution 3 is stable for 5 days at 2-8°C, for 3 weeks at -15 to -25°C.

Procedure
Wavelength: 340 or 365 nm
Temperature: 20-25°C
Read against air (without a cuvette in the light path) or against water or another (A2).

Mix**, read absorbances of the solutions (A1) after approx. 5 min. Start reaction by addition of:

Mix**, close the cuvettes*** and wait for the end of the reaction (20 min at 20-25°C). Read absorbances of blank and sample immediately one after another (A2).

Dilution table

If the measured absorbance difference (ΔA) is too low (e.g., <0.100), the sample solution should be prepared again (weigh out more sample or dilute less strongly) or the sample volume to be pipetted into the cuvette can be increased up to 2.000 ml. The volume of water added must then be reduced to obtain the same final volume in the assays for sample and blank. The new sample volume must be taken into account in the calculation.

The absorption maximum of NADH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; if spectralline photometers equipped with a mercury vapor lamp are used, measurements are taken at a wavelength of 365 nm or 334 nm.

If desired, disposable cuvettes may be used instead of glass cuvettes.

For example, with Parafilm (trademark of the American Can Company, Greenwich, Ct., USA)

Wavelength: 340 nm, Hg 365 nm or Hg 334 nm

Formula

\[
c = \frac{V \times MW \times \Delta A}{\epsilon \times d \times v \times 1000}
\]

V = final volume [ml]
v = sample volume [ml]
MW = molecular weight of the substance to be assayed [g/mol]
d = light path [cm]
ε = extinction coefficient of NADH at:
   - Hg 340 nm = 6.3 [l × mmol⁻¹ × cm⁻¹]
   - Hg 334 nm = 6.8 [l × mmol⁻¹ × cm⁻¹]
   - Hg 365 nm = 3.4 [l × mmol⁻¹ × cm⁻¹]

It follows for formic acid:

\[
c = \frac{3.050 \times 46.03}{1.404} \times \Delta A = 2.18 \times \Delta A \text{ [g formic acid/l sample solution]}
\]

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

1. Instructions for performance of assay
The amount of formic acid present in the assay has to be between 1 µg and 20 µg (measurement at 365 nm) or 0.4 µg and 10 µg (measurement at 340, 334 nm), respectively. In order to obtain a sufficient absorbance difference, the sample solution is diluted to yield a formic acid concentration between 0.04 and 0.2 g/l or 0.02 and 0.1 g/l, respectively.

2. Technical information
2.1 Materials for decalcification may contain formic acid. Therefore, glass ware used for production and storage of solutions for the determination of formic acid have to be rinsed carefully with tap water and with redist. water afterwards after decalcification. Formic acid in the reagents is responsible for a high blank absorbance difference.

2.2 The volatility of formic acid has to be taken into consideration when assay control solutions are produced. Therefore, formic acid is always pipetted under the surface of (weakly alkaline) solutions.

2.3 The reagents, especially NAD, have to be free of formic acid.

2.4 In carrying out the calculation, a clear indication should be given as to whether the results are to be given as formic acid (molar mass 46.03 g/mol) or as formate (molar mass 45.02 g/mol). (In enzymatic determinations, the formate ion is measured.)

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1 The absorption maximum of NADH is at 340 nm. On spectrophotometers, measurements are taken at the absorption maximum; if spectralline photometers equipped with a mercury vapor lamp are used, measurements are taken at a wavelength of 365 nm or 334 nm.
2 If desired, disposable cuvettes may be used instead of glass cuvettes.
3 For example by using a double-beam photometer
4 See instructions for performance of assay

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For in vitro use only
Store at 2-8°C

For recommendations for methods and standardized procedures see references (2)
3. Specificity (Ref. 1)  
The method is specific for formic acid. Acetic acid, propionic acid, oxalic acid and L-ascorbic acid do not influence the determination. Formaldehyde reduces the reaction rate but does not influence the specificity of the method.  
In the analysis of commercial formic acid results of < 100% have to be expected because formic acid is split into CO and water during storage. (The volatility of formic acid has to be considered when formic acid solutions are produced.)  

4. Sensitivity and detection limit (Ref. 1.2)  
The smallest differentiating absorbance for the procedure is 0.004 absorbance units. This corresponds to a maximum sample volume \( v = 2.000 \text{ ml} \) and measurement at 340 nm of an formic acid concentration of 0.05 mg/l sample solution (if \( v = 0.100 \text{ ml} \), this corresponds to 1 mg/l sample solution).  
The detection limit of 0.2 mg/l is derived from the absorbance difference of 0.020 (as measured at 340 nm) and a maximum sample volume \( v = 2.000 \text{ ml} \).  

5. Linearity  
Linearity of the determination exists from approx. 0.4 µg formic acid/assay (0.2 mg formic acid/l sample solution; sample volume \( v = 2.000 \text{ ml} \)) to 20 µg formic acid/assay (0.2 g formic acid/l sample solution; sample volume \( v = 0.100 \text{ ml} \)).  

6. Precision  
In a double determination using one sample solution, a difference of 0.005 to 0.010 absorbance units may occur. With a sample volume of \( v = 0.100 \text{ ml} \) and measurement at 340 nm, this corresponds to a formic acid concentration of approx. 1-2 mg/l. (If the sample is diluted during sample preparation, the result has to be multiplied by the dilution factor \( F \). If the sample is weighed in for sample preparation, e.g. using 1 g sample/100 ml = 0.010 absorbance units. This corresponds to a maximum sample volume \( v = 2.000 \text{ ml} \).  

7. Interference/sources of error  
7.1 Reducing substances in the sample can diminish the reaction rate. In the presence of 100 mg sulfur dioxide in the assay the reaction stops after approx. 40 min. SO\(_2\) can be destroyed by addition of 10 µl hydroponic peroxide (30%, w/v) to the assay.  
L-Ascorbic acid does not interfere with the assay - even in high amounts.  
7.2 In the presence of formaldehyde the formic acid reaction is inhibited. Small amounts of formaldehyde (5 µg/assay) diminish the reaction rate of the formic acid reaction. 10 µg formaldehyde give a small inhibition and 100 µg a strong inhibition of the enzyme FDH.  
7.3 Interferences of the assay by amines (e.g. fish components) have not been observed.  

8. Recognizing interference during the assay procedure  
8.1 If the conversion of formic acid has been completed according to the time given under "Procedure", it can be concluded in general that no interference has occurred.  
8.2 On completion of the reaction, the determination can be restarted by adding sodium formate (qualitative or quantitative): if the absorbance is altered subsequent to the addition of the standard material, this is also an indication that no interference has occurred.  
8.3 Operator error or interference of the determination through the presence of substances contained in the sample can be recognized by carrying out a double determination using two different sample volumes (e.g. 0.100 ml and 0.200 ml): the measured differences in absorbance should be proportional to the sample volumes used.  
When analyzing acidic samples, it is recommended that different quantities (e.g. 1 g and 2 g) be weighed into 100 ml volumetric flasks. The absorbance differences measured and the weights of sample used should be proportional for identical sample volumes.  
8.4 Possible interference caused by substances contained in the sample can be recognized by using an internal standard as a control: in addition to the sample, blank and standard determinations, a further determination should be carried out with sample and assay control solution in the same assay. The recovery can then be calculated from the absorbance differences measured.  
8.5 Possible interference of the determination can be recognized by carrying out a recovery test: the sample should be prepared and analyzed with and without added standard material. The additive should be recovered quantitatively within the error range of the method.  

9. Reagent hazard  
The reagents used in the determination of formic acid are not hazardous materials in the sense of the Hazardous Substances Regulations, the Chemicals Law or EC Regulation 67/548/EEC and subsequent alteration, supplementation and adaptation guidelines. However, the general safety measures that apply to all chemical substances should be adhered to.  
After use, the reagents can be disposed of with laboratory waste, but local regulations must always be observed. Packaging material can be disposed of in waste destined for recycling.  

10. General information on sample preparation  
In carrying out the assay:  
Use clear, colorless and practically neutral liquid samples directly, or after dilution according to the dilution table, and of a volume up to 2.000 ml;  
Filter turbid solutions; 
Degas samples containing carbon dioxide (e.g. by filtration);  
Adjust acid samples to pH 7-8 by adding sodium or potassium hydrogen solution;  
Adjust acid and weakly colored samples to pH 7-8 by adding sodium or potassium hydrogen solution and incubate for approx. 15 min;  
Measure "colored"samples (if necessary adjusted to pH 7-8) against a sample blank (= buffer or reagent, water + sample), adjust the photometer to 0.000 with the blank in the beam;  
Treat "strongly colored" samples that are used undiluted or with a higher sample volume with activated charcoal (e.g. 2 g/100 ml);  
Crush or homogenize solid or semi-solid samples, extract with water or dilute in water and filter necessary; alternatively remove turbidities and dyestuffs with Carrez reagents;  
Deproteinize samples containing protein with perchloric acid or with tri-chloroacetic acid; alternatively clarify with Carrez reagents.  

Carrez clarification:  
Pipe the liquid sample into a 100 ml volumetric flask which contains approx. 60 ml redist. water, or weigh sufficient quantity of the sample into a 100 ml volumetric flask and add approx. 60 ml redist. water. Subsequently, carefully add 5 ml Carrez-I-solution (potassium hexacyanoferrate(II) (ferrocyanide), 85 mM = 3.60 g K\(_4\)(CN)\(_6\) × 3 H\(_2\)O/100 ml) and 5 ml Carrez-II-solution (zinc sulfate, 250 mM = 7.20 g ZnSO\(_4\) × 7 H\(_2\)O/100 ml), Adjust to pH 7-8.5 with sodium hydroxide (0.1 M; e.g. 10 ml). Mix after each addition. Fill the volumetric flask to the mark, mix and filter.  

11. Application examples  
Determination of formic acid in fruit juices  
Dilute fruit juices with a high formic acid content with water in a ratio of 1+1; use 0.100 ml for the assay. When analyzing strongly colored fruit juices 100 mg charcoal are added to 5 ml juice. After ca. 1 min of stirring, filter. Adjust strongly acid juices to pH 7-8 with potassium hydrogen (1 M), if necessary (if high sample volumes are used).  
Determination of formic acid in mixed pickles  
Separate the liquid from the solid part, e.g. by filtration. For fat separation place the sample for 30 min in a refrigerator or for 20 min in an ice-bath, filter and dilute according to the dilution table.  
Determination of formic acid in wine  
Treat the sample as described under fruit juices. When analyzing red wine add 100 mg charcoal to 5 ml sample, stir for 1 min and filter. Use 0.200 ml for the assay.  
Determination of formic acid in vinegar  
Neutralize sample with potassium hydrogen (1 M) to pH 7-8. Dilute with water in a ratio of 1 + 1. Use 0.200 ml for the assay.  
Determination of formic acid in fruit and vegetable products  
Accurately weigh approx. 50 g homogenized vegetables into a 250 ml volumetric flask and add approx. 100 ml water. Stir the mixture for approx. 15 min (magnetic stirrer) in a closed flask. Fill up to the mark with water, mix, filter and centrifuge if necessary. Adjust strongly acidic sample solutions with a few drops of potassium hydroxide (1 M) to pH 7-8. Use 0.100 ml sample solution, diluted according to the dilution table, if necessary, for the assay.  
Determination of formic acid in fish and meat products  
Accurately weigh approx. 5 g homogenized sample into a homogenizer, add approx. 20 ml perchloric acid solution (1 M) and homogenize for 10 min. Transfer the mixture quantitatively into a beaker, add with potassium hydrogen (2 M) to pH 9-10 under stirring (magnetic stirrer). Transfer the mixture with approx. 20 ml water quantitatively into a 100 ml volumetric flask, fill up to the mark with redist. water. Take care that the fatty layer stands above the mark. Mix and place the mixture for separation of fat and water in a water bath at approx. 40 °C for 20 min in a refrigerator. Filter, discard the first few ml and use the clear slightly turbid solution for the assay. For calculation of the amount of formic acid take the volume displacement factor of 0.98 into account.
Formic acid assay control solution

The assay control solution serves as a control for the enzymatic determination of formic acid in foodstuffs and other materials.

Reagents
Sodium formate, AR grade

Preparation of the assay control solution

Accurately weigh approx. 148 mg sodium formate to the nearest 0.1 mg into a 1000 ml volumetric flask, fill up to the mark with redist. water, and mix thoroughly (this corresponds to approx. 0.1 g formic acid/l). Prepare assay control solution freshly before use. The assay control solution may be frozen in portions.

Application

1. Addition of formic acid assay control solution to the assay mixture:

   Instead of sample solution the assay control solution is used for the assay. (The measurement of the assay control solution is not necessary for calculating results.)

2. Restart of the reaction, quantitatively:

   After completion of the reaction with sample solution and measuring of A2, add 0.050 ml assay control solution to the assay mixture. Read absorbance A3 after the end of the reaction (approx. 20 min.). Calculate the concentration from the difference of (A2-A3) according to the general equation for calculating the concentration. The altered total volume must be taken into account. Because of the dilution of the assay mixture by the addition of the assay control solution, the result differs insignificantly from the result got according to pt. 1.

3. Internal standard:

   The assay control solution can be used as an internal standard in order to check the determination for correct performance (gross errors) and to see whether the sample solution is free from interfering substances.

<table>
<thead>
<tr>
<th>Pipette into cuvettes</th>
<th>Blank</th>
<th>Sample</th>
<th>Standard</th>
<th>Sample + Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>reaction mixture 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sample solution</td>
<td>-</td>
<td>1.000 ml</td>
<td>1.000 ml</td>
<td>1.000 ml</td>
</tr>
<tr>
<td>assay control sln.</td>
<td>-</td>
<td>0.100 ml</td>
<td>0.100 ml</td>
<td>0.050 ml</td>
</tr>
<tr>
<td>redist. water</td>
<td>-</td>
<td>1.900 ml</td>
<td>1.900 ml</td>
<td></td>
</tr>
<tr>
<td>Mix, and read absorbances of the solutions (A1) after approx. 5 min. Continue as described in the pipetting scheme under “Procedure.” Follow the instructions given under “Instructions for performance of assay” and the footnotes.</td>
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</tr>
</tbody>
</table>

\[
\text{recovery} = \frac{2 \times \Delta A_{\text{sample + standard}} - \Delta A_{\text{sample}}}{\Delta A_{\text{Standard}}} \times 100 \, [\%]
\]

4. Recovery experiments with original samples:

   For checking sample preparation and assay, recovery experiments may be carried out for this. For either, the a.m. assay control solution is used or another assay control solution with a suitable concentration is prepared. The original sample is measured with and without added sodium formate. The amount of added sodium formate
   - is either the same as expected to be present in the original sample,
   - or corresponds to that amount of formic acid which is allowed to be contained in the sample e.g. according to standards or other regulations.