FORMIC ACID (FORMATE)

PRODUCT INSTRUCTIONS

SKU: 700004282
K-FORM

09/25

(*25 Manual Assays per Kit) or
(220 Auto-Analyser Assays per Kit) or
(250 Microplate Assays per Kit)

** The number of tests per kit can be doubled if all volumes are halved*

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INTRODUCTION:

Formic acid is a naturally occurring organic acid with important applications across the food and beverage industry. It plays a key role in preserving the quality and safety of products such as fruit juices, wines, and other foodstuffs, thanks to its antimicrobial properties and regulatory approval as preservative E236.

This kit enables accurate detection and analysis of formic acid levels, supporting quality control, shelf-life optimization, and compliance with food safety standards..

PRINCIPLE:

In the presence of NAD^+, formic acid is oxidised to carbon dioxide (CO_2) by the enzyme formate dehydrogenase (FDH) (1) with the concurrent formation of NADH.

$$\text{(1) Formic acid} + NAD^+ \xrightarrow{\text{(FDH)}} CO_2 + NADH + H^+$$

The amount of NADH formed is stoichiometric with the amount of formic acid. It is the NADH which is measured by the increase in absorbance at 340 nm.

SPECIFICITY, SENSITIVITY, LINEARITY AND PRECISION:

The method is specific for formic acid. acetic acid, propionic acid, ascorbic acid and oxalic acid do not react.

The smallest differentiating absorbance for the assay is 0.005 absorbance units. This corresponds to 0.0466 mg/L of sample solution at the maximum sample volume of 2.00 mL (or 0.932 mg/L with a sample volume of 0.1 mL). The detection limit is 0.0932 mg/L, which is derived from an absorbance difference of 0.01 with the maximum sample volume of 2.00 mL.

Formic acid is split into carbon dioxide and water during storage and thus in the analysis of commercial formic acid recoveries of < 100% can be expected. The volatility of the solution must also be taken into consideration.

The assay is linear over the range of 0.4 to 20 µg of formic acid per assay. In duplicate determinations using one sample solution, an absorbance difference of 0.005 to 0.010 may occur. With a sample volume of 2.00 mL, this corresponds to a formic acid concentration of approx. 0.0466 to 0.0932 mg/L. If the sample is diluted during sample preparation, the result is multiplied by the dilution factor, F. If, in sample preparation, the sample is weighed, e.g. 10 g/L, a difference of 0.02 to 0.05 g/100 g can be expected.

INTERFERENCE:

If the conversion of formic acid has been completed within the specified time (approx. 12 min), it can generally be concluded that no interference has occurred. However, this can be further checked by adding formic acid (approx. 10 µg in 0.1 mL) to the cuvette on completion of the reaction. A significant increase in the absorbance should be observed.

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.

Formate dehydrogenase is inhibited by sodium azide, so this should be excluded from all assay components and samples.

SAFETY:

The general safety measures that apply to all chemical substances should be adhered to.

For more information regarding the safe usage and handling of this product please refer to the associated SDS that is available from the Megazyme® and Neogen® websites.

KITS:

Kits suitable for performing 25 assays in manual format (or 220 assays in auto-analyser format or 250 assays in microplate format) are available from Neogen. The kit contains:

Bottle 1: (x2) Buffer (freeze-dried powder, pH 7.6).
Store below -10°C. See individual label for expiry.

Bottle 2: NAD^+.
Store below -10°C. See individual label for expiry.

Bottle 3: Formate dehydrogenase (1.4 mL).
Store at 4°C. See individual label for expiry.

Bottle 4: Sodium formate (~ 2 g).
Store at 4°C. See individual label for expiry.

PREPARATION OF REAGENT SOLUTIONS/SUSPENSIONS:

1. Dissolve the contents of one of bottle 1 in 4 mL of distilled water. Store below -10°C between use. Stable for 2 years below -10°C. Do not dissolve the contents of the second bottle until required. This is **buffer solution**.

2. Dissolve the contents of bottle 2 in 5.2 mL of distilled water. Divide into appropriately sized aliquots and store in polypropylene tubes below -10°C between use and keep cool during use if possible. Once dissolved, the reagent is stable for 2 years below -10°C. This is **NAD^+ solution**.

3. Use the contents of **bottle 3** as supplied. Before opening for the first time, shake the bottle to remove any enzyme that may have settled on the rubber stopper. Subsequently, store the bottle in an upright position. **Swirl the bottle to mix contents before use.**

NOTE: The sodium formate standard solution is only assayed where there is some doubt about the accuracy of the spectrophotometer being used or where it is suspected that inhibition is being caused by substances in the sample. The concentration of formic acid is determined directly from the extinction coefficient of NADH (see page 5).

4. Accurately weigh 148 mg of sodium formate (MW = 68.08) to the nearest 0.1 mg into a 1 L volumetric flask. Fill to the mark with distilled water and mix thoroughly. This corresponds to 0.1 g/L formic acid. Divide into appropriately sized aliquots and store below -10°C. Stable for 1 year below -10°C.

EQUIPMENT (RECOMMENDED):

1. Glass test tubes (round bottomed; 16 x 100 mm).
2. Disposable plastic cuvettes (1 cm light path, 3.0 mL).
3. Micro-pipettors, e.g. Gilson® Pipetman® (20 µL, 100 µL and 200 µL).
4. Positive displacement pipettor, e.g. Eppendorf® Multipette®
   - with 5.0 mL Combitip® (to dispense 0.2 mL aliquots of NAD^+ solution).
   - with 25 mL Combitip® (to dispense 2.0 mL aliquots of distilled water).
5. Analytical balance.
6. Spectrophotometer set at 340 nm.
7. Vortex mixer.
8. Stop clock.
9. Whatman® No. 1 (9 cm) filter papers.

A. MANUAL ASSAY PROCEDURE:

Wavelength: 340 nm
Cuvette: 1 cm light path (glass or plastic)
Temperature: ~ 25°C
Final volume: 2.55 mL
Sample solution: 0.4-20 µg of formic acid per cuvette (in 0.10-2.00 mL sample volume)
Read against air (without a cuvette in the light path) or against water

Pipette into cuvettes	Blank	Sample
distilled water (at ~ 25°C)	2.10 mL	2.00 mL
sample*	-	0.10 mL
buffer solution	0.20 mL	0.20 mL
NAD^+ solution	0.20 mL	0.20 mL
Mix**, read the absorbances of the solutions (A_1) after approx. 5 min and start the reactions by addition of:		
bottle 3 (FDH)	0.05 mL	0.05 mL
Mix** and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 12 min).		

* rinse the dispensing pipette tip with the sample solution before dispensing the sample.
** for example with a plastic spatula or by gentle inversion after sealing the cuvette with a cuvette cap or Parafilm®.

CALCULATION:

Determine the absorbance difference (A_2-A_1) for both blank and sample. Subtract the absorbance difference of the blank from the absorbance difference of the sample, thereby obtaining $\Delta A_{formic\ acid}$. The value of $\Delta A_{formic\ acid}$ should as a rule be at least 0.100 absorbance units to achieve sufficiently accurate results.

The concentration of formic acid can be calculated as follows:

$$c = \frac{V \times MW}{\varepsilon \times d \times v} \times \Delta A_{formic\ acid} \quad [g/L]$$

where:

V = final volume [mL]
MW = molecular weight of formic acid [g/mol]
Ɛ = extinction coefficient of NADH at 340 nm
= 6300 [l x mol^{-1} x cm^{-1}]
d = light path [cm]
v = sample volume [mL]

It follows for formic acid:

$$c = \frac{2.55 \times 46.03}{6300 \times 1.0 \times 0.1} \times \Delta A_{formic\ acid} \quad [g/L]$$

$$= 0.1863 \times \Delta A_{formic\ acid} \quad [g/L]$$

If the sample has been diluted during preparation, the result must be multiplied by the dilution factor, F.

When analysing solid and semi-solid samples which are weighed out for sample preparation, the content (g/100 g) is calculated from the amount weighed as follows:

Content of formic acid

$$= \frac{c_{formic\ acid}\ [g/L\ sample\ solution]}{weight_{sample}\ [g/L\ sample\ solution]} \times 100 \quad [g/100\ g]$$

NOTE: These calculations can be simplified by using the Megazyme ***Mega-Calc***™, downloadable from where the product appears on the Megazyme website (www.megazyme.com).

B. AUTO-ANALYZER ASSAY PROCEDURE:

NOTES:

1. The Auto-Analyzer Assay Procedure for formic acid can be performed using either a single point standard or a full calibration curve.
2. For each batch of samples that is applied to the determination of formic acid **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**
3. Reagent R2 may appear slightly turbid, particularly after storage. This does not affect assay performance. If preferred, the reagent may be clarified by centrifugation at 15,000 g for 5 min.

Reagent preparation is performed as follows:

Preparation of R1:

Component	**Volume**
distilled water **buffer solution** **NAD^+ solution**	22.2 mL 2.5 mL 2.5 mL (after adding 5.2 mL of H_2O to bottle 2)
Total volume	27.2 mL

Preparation of R2:

Component	**Volume**
distilled water **bottle 3** (FDH)	2.30 mL 0.51 mL
Total volume	2.81 mL

EXAMPLE METHOD:

R1:	0.200 mL
Sample:	~ 0.01 mL
R2:	0.025 mL
Reaction time:	~ 12 min at 37°C
Wavelength:	340 nm
Prepared reagent stability:	2 days when refrigerated
Calculation:	endpoint
Reaction direction:	increase
Linearity:	up to 0.183 g/L of formic acid using 0.01 mL sample volume

C. MICROPLATE ASSAY PROCEDURE:

NOTES:

1. The Microplate Assay Procedure for formic acid can be performed using either a single point standard or a full calibration curve.
2. For each batch of samples that is applied to the determination of formic acid **either a single point standard or a calibration curve must be performed concurrently using the same batch of reagents.**

Wavelength: 340 nm
Microplate: 96-well (e.g. clear flat-bottomed, glass or plastic)
Temperature: ~ 25°C
Final volume: 0.255 mL
Linearity: 0.04-2.0 µg of formic acid per well (in 0.01-0.20 mL sample volume)

Pipette into wells	Blank	Sample	Standard
distilled water	0.210 mL	0.200 mL	0.200 mL
sample solution	-	0.010 mL	-
standard solution	-	-	0.010 mL
buffer solution	0.020 mL	0.020 mL	0.020 mL
NAD^+ solution	0.020 mL	0.020 mL	0.020 mL
Mix*, read the absorbances of the solutions (A_1) after approx. 5 min and start the reactions by addition of:			
bottle 3 (FDH)	0.005 mL	0.005 mL	0.005 mL
Mix* and read the absorbances of the solutions (A_2) at the end of the reaction (approx. 12 min). If the reaction has not stopped after 12 min, continue to read the absorbances at 2 min intervals until the absorbances increase constantly over 2 min**.			

* for example using microplate shaker, shake function on a microplate reader or repeated aspiration (e.g. using a pipettor set at 50-100 µL volume).

** if this "creep" rate is greater for the sample than for the blank, extrapolate the sample absorbances back to the time of addition of suspension 3.

CALCULATION (Microplate Assay Procedure):

$$g/L = \frac{\Delta A_{sample}}{\Delta A_{standard}} \times g/L \text{ standard} \times F$$

If the sample is diluted during preparation, the result must be multiplied by the dilution factor, F.

SAMPLE PREPARATION:

1. Sample dilution.

The amount of formic acid present in the cuvette (i.e. in the 0.1 mL of sample being analysed) should range between 0.4 and 20 µg. The sample solution must therefore be diluted sufficiently to yield a formic acid concentration between 0.004 and 0.20 g/L.

Dilution Table

Estimated concentration of formic acid (g/L)	Dilution with water	Dilution factor (F)
< 0.2	No dilution required	1
0.2-2.0	1 + 9	10
2.0-20	1 + 99	100
> 20	1 + 999	1000

If the value of $\Delta A_{formic\ acid}$ is too low (e.g. < 0.100), weigh out more sample or dilute less strongly. Alternatively, the sample volume to be pipetted into the cuvette can be increased up to 2.00 mL, making sure that the sum of the sample and distilled water components in the reaction is 2.10 mL and using the new sample volume in the equation.

NOTE: The below are suggested sample preparation examples only. Users should perform in-house matrix validation work prior to routine use. This process will highlight any problematic matrices encountered.

2. Sample clarification.

Samples containing high amounts of protein or fats may cause interference in target analyte determination and require sample clarification prior to analysis using Carrez Clarification reagents. These reagents are available to purchase separately from Neogen in the Carrez Clarification Kit (**K-CARREZ, 700004270**). The **K-CARREZ** reagents should be diluted prior to use as described on page 2 of the **K-CARREZ** assay protocol and the assay procedure for clarification followed as described on page 3 of **K-CARREZ** instructions. The clarified sample solution prepared using the **K-CARREZ** assay kit can then be analysed as described in the assay procedure.

3. General considerations.

(a) Liquid samples: clear, slightly coloured and approximately neutral, liquid samples can be used directly in the assay.

(b) Acidic samples: if > 0.1 mL of an acidic sample is to be used undiluted (such as wine or fruit juice), the pH of the solution should be increased to approx. 8.0 using 2 M NaOH, and the solution incubated at room temperature for 30 min.

(c) Carbon dioxide: samples containing significant quantities of carbon dioxide, such as beer, should be degassed by increasing the pH to approx. 8.0 with 2 M NaOH and gentle stirring, or by stirring with a glass rod.

(d) Coloured samples: an additional sample blank, i.e. sample with no FDH, may be necessary in the case of coloured samples.

(e) Strongly coloured samples: if used undiluted, strongly coloured samples should be treated by the addition of 0.2 g of PVPP/10 mL of sample. Shake the tube vigorously for 5 min and then filter through Whatman No. 1 filter paper.

(f) Solid samples: homogenise or crush solid samples in distilled water and filter if necessary.

(g) Samples containing fat: extract such samples with hot water at a temperature above the melting point of the fat, e.g. in a 100 mL volumetric flask. Adjust to room temperature and fill the volumetric flask to the mark with distilled water. Store on ice or in a refrigerator for 15-30 min and then filter. Discard the first few mL of filtrate, and use the clear supernatant (which may be slightly opalescent) for assay. Alternatively, clarify with **K-CARREZ** reagents.

(h) Samples containing protein: deproteinise samples containing protein by adding an equal volume of ice-cold 1 M perchloric acid with mixing. Centrifuge at 1,500 *g* for 10 min and neutralise the supernatant with 1 M KOH. Alternatively, use **K-CARREZ** reagents.

SUGGESTED SAMPLE PREPARATION EXAMPLES:

(a) Determination of formic acid in wine.
For red wine, add 100 mg of charcoal to 5 mL of sample in a polypropylene tube. Mix by inversion for 1 min and then filter the suspension through Whatman GF/A glass fibre filter paper. For white wine, analyse the sample with no prior pre-treatment. *Typically, no dilution is required and a sample volume of 0.1 mL is satisfactory.*

(b) Determination of formic acid in fruit juice.
For strongly coloured juices, add 100 mg of charcoal to 5 mL of sample in a polypropylene tube. Mix by inversion for 1 min and then filter the suspension through Whatman GF/A glass fibre filter paper. Adjust strongly acidic juices to pH 7-8 using 1 M potassium hydroxide. *Typically, no dilution is required and a sample volume of 0.1 mL is satisfactory.*

(c) Determination of formic acid in vinegar.
Add 50 mL of vinegar to a 200 mL beaker and adjust to pH 7-8 with 1 M potassium hydroxide. Quantitatively transfer to a 100 mL volumetric flask and adjust to the mark with distilled water. *Typically, no further dilution is required and a sample volume of 0.2 mL is satisfactory.*

(d) Determination of formic acid in pickles.
Separate liquid from solid by filtration. Store the solution at 4°C for 20 min to obtain separation of fat (if necessary). Filter an aliquot of the aqueous layer, discarding the first few mL. Dilute an aliquot of the filtrate according to the dilution table, if necessary. *Typically, no dilution is required and a sample volume of 0.1 mL is satisfactory.*

(e) Determination of formic acid in fruit and vegetable products.
Homogenise approx. 50 g of fruit or vegetable with 100 mL of water using an electric blender. Stir the mixture for approx. 15 min and quantitatively transfer to a 250 mL volumetric flask and adjust to the mark with distilled water. Mix well. *Typically, no dilution is required and a sample volume of 0.2 mL is satisfactory.*

(f) Determination of formic acid in fish and meat products. Accurately weigh approx. 5 g of representative homogenised sample into a 50 mL beaker. Add 20 mL perchloric acid solution (1 M) and, using a Polytron® homogeniser or similar, homogenise for 10 min. Adjust the pH to approx. 8 with 2 M potassium hydroxide solution. Quantitatively transfer the mixture to a 100 mL volumetric flask and adjust to the mark with distilled water, ensuring the fatty layer is "above" the mark and that the aqueous layer is "at" the mark. Filter the solution through Whatman No. 1 (9 cm) filter paper. Discard the first few mL and use the clear or slightly turbid solution for the assay. For calculation of the amount of formic acid, take the volume displacement factor of 0.98 into account. *Typically, no dilution is required and a sample volume of 0.5 mL is satisfactory.*

(g) Determination of formic acid in bakery goods.
Accurately weigh 5 g of homogenised or milled sample into a 100 mL volumetric flask. Add approx. 75 mL of distilled water and extract at 20-25°C for 15 min. Fill to the mark with distilled water and filter the solution through Whatman No. 1 (9 cm) filter paper. *Typically, no dilution is required and a sample volume of 0.2 mL is satisfactory.*

(h) Determination of formic acid in honey.
Weigh approx. 10 g of honey into a 100 mL volumetric flask. Fill to the mark with distilled water and mix thoroughly. Use the solution directly in the assay. *Typically, no dilution is required and a sample volume of 0.2 mL is satisfactory.*

(i) Determination of formic acid in jam.
Accurately weigh approx. 20 g of homogenised sample into a 100 mL beaker. Add approx. 20 mL of hot water (~ 60°C) and mix. Then add 2 mL of Carrez I solution and 2 mL of Carrez II solution. Mix after each addition. Neutralise the sample with 4 mL of 100 mM NaOH. Cool the solution to 20-25°C, quantitatively transfer to a 100 mL volumetric flask and fill to mark with distilled water. Mix thoroughly and filter through Whatman No. 1 (9 cm) filter paper. *Typically, no dilution is required and a sample volume of 0.2 mL is satisfactory.*

(j) Determination of formic acid in protein containing samples.

Add 40 mL of 30 mM trichloroacetic acid to 20 g of protein- containing sample and stir the mixture for 1 min. Neutralise the solution with 1 M potassium hydroxide and quantitatively transfer the mixture to a 100 mL volumetric flask. Fill to mark with distilled water and filter an aliquot through Whatman No. 1 (9 cm) filter paper. Use the clear solution, diluted if necessary, in the assay. *Typically, no dilution is required and a sample volume of 0.1 mL is satisfactory.*

NOTE: If you have questions about these or other matrices, please contact your local sales representative for support.

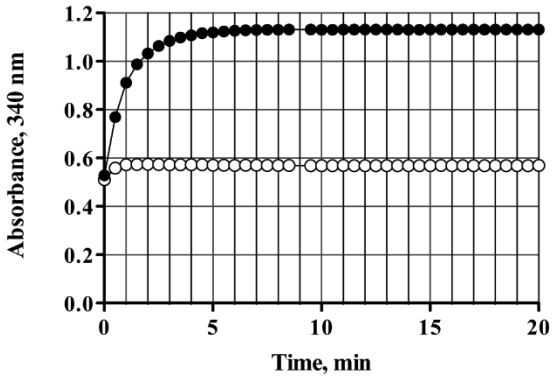

Figure 1. Increase in absorbance at 340 nm on incubation of 10 µg of formic acid with formate dehydrogenase in the presence of NAD^+.

REFERENCE:

Schaller, K. -H. & Triebig, G. (1988). Formate: Determination with Formate Dehydrogenase. "*Methods of Enzymatic Analysis*" (Bergmeyer, H. U., ed.), 3rd ed., **Vol VI**, pp. 668-672, VCH Publishers (UK) Ltd., Cambridge, UK.

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