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Product Information

Formate Assay Kit

Catalog Number **MAK059**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description
Formate, the salt form of formic acid, is the simplest carboxylate anion. Formate is produced in the mitochondria as a result of the oxidation of one-carbon donors such as serine. Mitochondrially-derived formate is utilized for cytoplasmic one-carbon metabolism in the *de novo* synthesis of purines, thymdiylate, and the remethylation of homocysteine to methionine. Under normal physiologic conditions, formate is present at low levels in serum and blood but levels may be increased following acute methanol poisoning or environmental exposure to formaldehyde. Serum and urine formate levels were increased in vitamin B_{12}-deficient rats and may be indicative of hyperhomocysteinemia due to defects in remethylation.

The Formate Assay Kit provides a simple, sensitive, and rapid means of quantifying formate in a variety of samples including serum, urine, media, cells, and tissues. Formate concentrations are determined by an enzymatic assay, which results in a colorimetric (450 nm) product, proportional to the formate present.

Components
The kit is sufficient for 100 assays in 96 well plates.

Formate Assay Buffer Catalog Number MAK059A	25 mL
Formate Enzyme Mix Catalog Number MAK059B	1 vl
Formate Substrate Mix Catalog Number MAK059C	1 vl
Formate Standard, 100 mM Catalog Number MAK059D	0.1 mL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Formate Assay Buffer – Allow buffer to come to room temperature before use.

Formate Enzyme Mix – Reconstitute in 220 µL of Formate Assay Buffer. Mix well by pipetting, then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Formate Substrate Mix – Reconstitute in 220 µL of water. Mix well by pipetting, then store at 4 °C. Use within 2 months of reconstitution.

Storage/Stability
The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Formate Standards for Colorimetric Detection
Dilute 10 µL of the 100 mM Formate Standard Solution with 990 µL of water to generate a 1 mM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 1 mM formate standard into a 96 well plate, generating 0 (blank), 2, 4, 6, 8, and 10 nmole/well standards. Add Formate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (10 mg) or cells (1×10^6) should be rapidly homogenized with 100 µL of the Formate Assay Buffer. Centrifuge the samples at 15,000 × *g* for 10 minutes to remove insoluble material. Bring samples to a final volume of 50 µL with Formate Assay Buffer.

Serum samples (0.5–10 µL/well) can be added directly to well and brought to a final volume of 50 µL/ well with Formate Assay Buffer.

For unknown samples, it is suggested to test several sample volumes to make sure the readings are within the standard curve range.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1.
   Note: NADPH present in samples can cause background in the assay. To remove the NADPH background, include a blank sample for each sample by omitting the Formate Enzyme Mix. The background control readings can then be subtracted from the sample readings.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
Formate Assay Buffer	46 µL	48 µL
Formate Enzyme Mix	2 µL	–
Formate Substrate Mix	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the standard, sample, and blank control wells. Mix well using a horizontal shaker or by pipetting, and incubate at 37°C for 60 minutes, protected from light.

3. Measure the absorbance at 450 nm (A_{450}).

Results
Calculations
The background for the assay is the value obtained for the 0 (blank) Formate standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings.

Use the values obtained from the formate standards to plot a standard curve. The amount of formate present in the samples may be determined from the standard curve.
Note: A new standard curve must be set up each time the assay is run.

Concentration of Formate

$$S_a/S_v = C$$

S_a = Amount of formate in unknown sample (nmole) from standard curve
S_v = Sample volume (µL) added into the wells.
C = Concentration of formate in sample

Formic Acid molecular weight: 46.02 g/mole

Sample Calcualtion
Formate amount (S_a) = 5.84 nmole
Assay volume (S_v) = 50 µL

$$5.84 \text{ nmole}/50 \text{ µl} = 0.1169 \text{ nmole}/\text{µL}$$

$$0.1169 \text{ nmole}/\text{µL} \times 46.02 \text{ ng/nmole} = 5.38 \text{ ng}/\text{µL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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