EnzyChrom™ Formate Assay Kit (EFOR-100)

Quantitative Colorimetric Formate Determination

DESCRIPTION

FORMATE ($HCOO^-$) is the anion derived from formic acid, the simplest carboxylic acid. It is also the metabolic byproduct of formaldehyde metabolism in our body, and the eventual metabolic byproduct of methanol which is first broken down to formaldehyde. At high levels, formate is neurotoxic to the central nervous system and can cause blindness, coma, and death. Although naturally present in the body at low levels, elevated levels of formate may be used as an indicator of formaldehyde exposure and methanol poisoning.

BioAssay Systems' formate assay kit is based on formate dehydrogenase catalyzed oxidation of formate, which generates carbon dioxide and NADH that reduces a formazan (MTT) dye. The intensity of the reduced MTT, measured at 565 nm, is directly proportional to formate concentration in the sample.

KEY FEATURES

Fast and sensitive. Use of 10 µL sample. Linear detection range 0.050 to 5 mM Formate in 96-well plate assay.

Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 60 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: formate in biological samples such as urine and serum.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**MTT/NAD:**	1 mL
Enzyme A:	120 µL	**Standard:**	0.5 mL 20 mM Formate
Enzyme B:	120 µL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation: clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor *n*.

Biological fluid samples (e.g. urine & serum) can be assayed directly, after centrifuging first to remove any particulates. Appropriate dilution in distilled water may be required.

Procedure using 96-well plate

1. *Standards*. Prepare 200 µL 1 mM Premix by mixing 10 µL of the Standard (20 mM) and 190 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H_2O	Formate (mM)
1	100 µL + 0 µL	1.0
2	60 µL + 40 µL	0.6
3	30 µL + 70 µL	0.3
4	0 µL + 100 µL	0

2. Transfer 10 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 10 µL of each sample into separate wells.
3. Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 85 µL Assay Buffer, 8 µL MTT/NAD, 1 µL Enzyme A, and 1 µL Enzyme B.

   Add 90 µL WR to the *four Standards* and the *Sample Wells*. Tap plate to mix briefly and thoroughly. Incubate 60 minutes at room temperature.
4. Read optical density at 565 nm (520-600 nm).

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the formate concentration of Sample,

$$[\text{Formate}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (mM^{-1})} \times n \quad (mM)$$

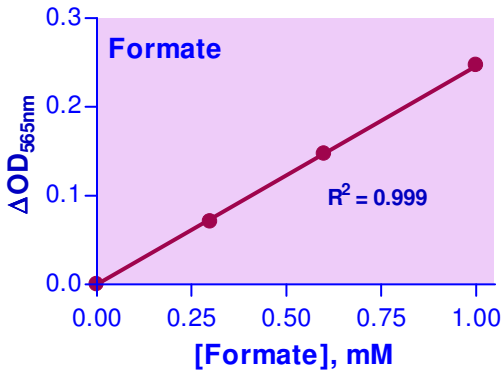
OD_{SAMPLE} and OD_{BLANK} are optical density readings of the Sample and Water Blank (#4), respectively. *n* is the sample dilution factor.

Note: if the sample OD value is higher than OD for the 1 mM formate standard, dilute sample in water and repeat the assay. Multiply the results by the dilution factor.

Conversions: 1 mM formate equals 4.5 mg/dL, or 45 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



Standard Curve in 96-well plate assay in water

LITERATURE

1. Kerns II, William, et al (2002). Formate Kinetics in Methanol Poisoning. J Toxicol Clin Toxicol 40.2: 137-143.
2. Martin-Amat, G., et al (1978). Methanol poisoning: Ocular toxicity produced by formate. Toxicol Appl Pharm 45.1: 201-208.
3. Boeniger, M. F. (1987). Formate in Urine as a Biological Indicator of Formaldehyde Exposure: A Review. Neurogastroenterology Am Ind Hyg Assoc J 48.11: 900-908.