Formate Assay Kit (Colorimetric)

LS-K315-100 (100 Tests) • Store at -20°C



Introduction

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.

LSBio's 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.

Components

| | K315-100 | |
|--------------------------|-----------|--|
| Component | 100 Tests | |
| Assay Buffer | 10 mL | |
| Enzyme A | 120 μL | |
| Enzyme B | 120 μL | |
| MTT/NAD | 2 mL | |
| Standard (20 mM Formate) | 0.5 mL | |

Materials Not Supplied

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

Storage

The kit is shipped on ice. Store all kit components at -20 °C.

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Assay Procedure

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

| No | Premix + H ₂ O | 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 μ L standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 10 μ L of each sample into separate wells.
- 3. Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 85 μ L Assay Buffer, 8 μ L MTT/NAD, 1 μ L Enzyme A, and 1 μ L Enzyme B.

Add 90 μ L 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).

Calculations

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

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

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.

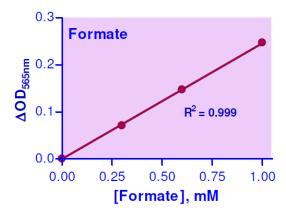
Conversions: 1 mM formate equals 4.5 mg/dL, or 45 ppm.

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Sample Data



Standard Curve in 96-well plate assay in water

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